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A GUIDE TO...

A guide to COVID-19 antiviral therapeutics: a summary and perspective of the antiviral weapons against SARS-CoV-2 infection

Drugan K. Brady, Aashi R. Gurijala, Liyu Huang, Ali A. Hussain, Audrey L. Lingan, Olivia G. Pembridge, Brina A. Ratangee, Tristan T. Sealy, Kyle T. Vallone and Thomas P. Clements

Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA

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Correspondence
T. P. Clements, Department of Biological Sciences, Vanderbilt University, 1210 Biological Sciences/MRB, 465 21st Street South, Nashville, TN 37235, USA
Tel: +1 615 343 5477
E-mail: thomas.clements@vanderbilt.edu

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Antiviral therapies are integral in the fight against SARS-CoV-2 (i.e. severe acute respiratory syndrome coronavirus 2), the causative agent of COVID-19. Antiviral therapeutics can be divided into categories based on how they combat the virus, including viral entry into the host cell, viral replication, protein trafficking, post-translational processing, and immune response regulation. Drugs that target how the virus enters the cell include: Evusheld, REGEN-COV, bamlanivimab and etesevimab, bebtelovimab, sotrovimab, Arbidol, nitazoxanide, and chloroquine. Drugs that prevent the virus from replicating include: Paxlovid, remdesivir, molnupiravir, favipiravir, ribavirin, and Kaletra. Drugs that interfere with protein trafficking and post-translational processing include nitazoxanide and ivermectin. Lastly, drugs that target immune response regulation include interferons and the use of anti-inflammatory drugs such as dexamethasone. Antiviral therapies offer an alternative solution for those unable or unwilling to be vaccinated and are a vital weapon in the battle against the global pandemic. Learning more about these therapies helps raise awareness in the general population about the options available to them with respect to aiding in the reduction of the severity of COVID-19 infection. In this 'A Guide To' article, we provide an in-depth insight into the development of antiviral therapeutics against SARS-CoV-2 and their ability to help fight COVID-19.

Introduction

With the emergence of the COVID-19 pandemic in March 2020 came the challenge of synthesizing novel therapeutics to combat the spread and reduce the lethality of the virus that causes it: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Previous therapies used to treat HIV and Ebola virus

Abbreviations
$3CL^{pro}$, 3-chymotrypsin-like protease; ACE2, angiotensin-converting enzyme 2; BNE, bamlanivimab and etesevimab; EUA, emergency use authorization; FDA, United States Food and Drug Administration; FRTP, favipiravir-ribofuranosyl-5′-triphosphate; IFN, interferon; IgG1κ, human immunoglobulin G1; IMP, inosine monophosphate; MERS-CoV, Middle East respiratory syndrome coronavirus; M^{pro}, severe acute respiratory syndrome coronavirus 2 main protease; NHC, β-D-N4-hydroxycytidine; NIH, National Institutes of Health; NSP, non-structural protein; NTP, nucleotide triphosphate; NTZ, nitazoxanide; PDB, Protein Data Bank; PDI, protein disulfide isomerase; RBD, receptor binding domain; RdRp, RNA-dependent RNA polymerase; RMP, remdesivir monophosphate; RTP, remdesivir triphosphate; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; STAT, signal transducers and activators of transcription; TIZ, tizoxanide; WHO, World Health Organization.

disease show promise in targeting related mechanisms in SARS-CoV-2. Almost 180 vaccines against SARS-CoV-2 have undergone clinical development across the globe, yet only 11 have been approved for emergency use by the World Health Organization (WHO) [2]. Vaccine development is a lengthy and exhaustive process, relying on effective distribution in a systematic, regulated manner to reach as many individuals as possible. Without proper distribution methods, the vaccine ceases to accomplish its function of attaining herd immunity and protecting a community from disease outbreaks. It is also worth noting that vaccination of immunocompromised individuals is not as efficacious as in immunocompetent counterparts [3]. Therefore, antiviral treatments are actively being developed as another measure to fight COVID-19.

Antiviral therapies have numerous benefits, including the ability to be distributed to wide populations and administered to individuals who are immunodeficient. Additionally, negative perception of vaccines has led to distrust and vaccine refusal [4]. Therefore, antiviral therapeutics broaden the options for treatments and preventative measures.

In this 'A Guide To' article, we describe the mechanisms that the SARS-CoV-2 virus exploits as it infects the human body. We then discuss how antiviral therapeutics are used to stop its progression at each of these crucial steps. Each category of antiviral therapeutic [entry obstruction via S protein targeting (Fig. 1.1), interference with viral replication (Fig. 1.2–5), protein trafficking and post-translational modifications (Fig. 1.6–7), and immune response upregulation] will be discussed as we look at therapeutic treatments.

SARS-CoV-2 initiates infection using a viral spike protein (S protein) which binds to the angiotensin-converting enzyme 2 (ACE2) receptor of a host cell, triggering endocytosis of the virus (Fig. 1.1) [7]. Once endocytosed, the outer protein coat of the virus is shed, releasing viral contents into the host cell (Fig. 1.2). The genomic RNA of the virus is then translated by host cell ribosomes, followed by processing of the resulting proteins with viral proteases (Fig. 1.3–4). The 3-chymotrypsin-like cysteine protease of SARS-CoV-2 processes large viral polypeptides into proteins necessary for viral replication, which then form elements of the replication-transcription complex (Fig. 1.4) [5]. RNA-dependent RNA polymerase (RdRp), a major component of the replication-transcription complex, fulfills an essential role in the replication mechanism of SARS-CoV-2 (Fig. 1.5). The replicated RNA strands are then translated and structural and accessory viral proteins are processed, assembled, and receive post-translational modifications at the Golgi apparatus (Fig. 1.7). Finally, the virus with its fully assembled proteins forms an exocytic vesicle when budding with the cell membrane and is released through exocytosis from the cell (Fig. 1.8–9) [5].

Viral entry into host cell

The treatments discussed in this section target the first step of the viral life cycle (i.e. entry into the host cell) through one of two mechanisms. The first mechanism involves spike protein targeting, in which the drug binds to the S protein of SARS-CoV-2 and prevents it from forming interactions with the ACE2 receptor that are necessary for viral uptake. The second mechanism is endosomal targeting that creates an alkaline environment within endosomes/lysosomes, lowering cytokine levels. This mechanism disrupts endocytosis and thus does not allow the virus to enter the cell [8]. A summary of these treatments and their timing is found in Table 1.

Spike protein targeting

Evusheld

Evusheld was developed by AstraZeneca as a SARS-CoV-2 preventative treatment for adults and children (aged at least 12 years old and weighing 40 kg). The drug consists of two consecutive intramuscular injections of monoclonal antibodies tixagevimab and cilgavimab, preferably with one injection into each gluteal [9]. Originating from research carried out by the Vanderbilt University Medical Center in 2020, these antibodies were isolated from patients with COVID-19 and neutralized the S protein of SARS-CoV-2 *in vitro* [21].

The antibodies are classified as SARS-CoV-2 S protein-directed attachment inhibitors, comprising a type of human immunoglobulin G1 (IgG1κ) monoclonal antibody with amino acid substitutions to extend half-life. These antibodies simultaneously bind to separate regions of the receptor-binding domain (RBD) of the SARS-CoV-2 S protein to block its interaction with ACE2 (Fig. 2) [23]. Both monoclonal antibodies have a heavy and light chain, with the interface between the heavy/light chains of tixagevimab forming an aromatic cage. By contrast, cilgavimab has a long light chain and a heavy chain that is able to interact with the side opposite of tixagevimab on the RBD [21]. This means that, when used in tandem, the two antibodies provide complementary protection against the binding of the S protein to the host cell, thereby preventing entry.

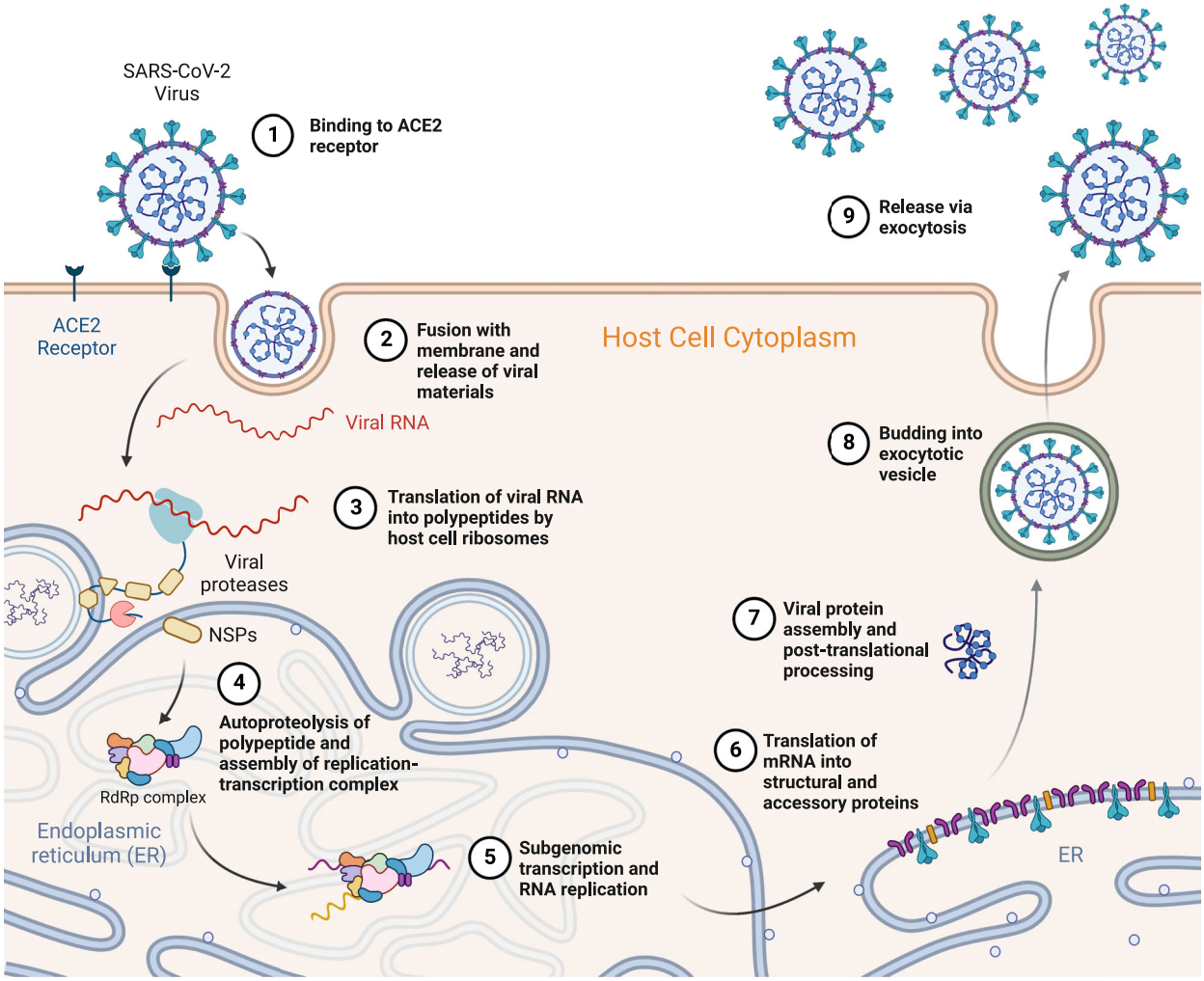

Fig. 1. Summarized pathway of the entry into host cell, replication, maturation, and release of the SARS-CoV-2 virus. (1) Entry and binding of S protein to the ACE2 receptor. (2) Shedding of the virus's outer endosome coat and fusion with the cellular membrane releases viral materials. Host factors allow for fusion of the endosomal and cellular membranes. (3) Translation of viral mRNA into polypeptides and viral proteases by host cell occurs. The polyproteins are post-translationally processed to form non-structural proteins (NSPs). (4) Autoproteolysis of the virus's translated proteins and assembly of the replication-transcription complex (RdRp complex). NSPs form the replication-transcription complex after processing. (5) Subgenomic transcription of host cell DNA into RNA and RNA replication occurs. The integrated viral genetic material is also processed at this step. (6) Translation of host cell mRNA and the integrated viral cell mRNA into structural and accessory proteins occurs on the ribosomes of the RER. Translocation of proteins occurs here as well. (7) Viral proteins are assembled and processed post-translation. This helps form secretory vesicles for exocytosis out of the host cell. (8) Viral RNA instructions cause the formation of exocytic vesicles. (9) Virus is released via exocytosis from the host cell [5]. Figure created with BioRender.com and adapted from 'Life Cycle of Coronavirus', by BioRender.com [6].

There are two ongoing phase 3 clinical trials: PROVENT [24] and STORM CHASER [25]. PROVENT used a randomized, double-blind trial with 5172 subjects who tested negative for SARS-CoV-2, with 3441 individuals receiving Evusheld and another 1731 receiving a placebo. Data collected after 83 days of treatment demonstrated a 77% risk reduction for contracting SARS-CoV-2, which was statistically significant compared to the placebo. In a follow-up 6.5 months after treatment, there was an 83% risk reduction for symptomatic illness [24]. Similarly, the STORM CHASER trial treated its 1121 subjects at a 2 : 1 ratio of Evusheld to saline placebo, resulting in a 33% relative risk reduction [25]. Observed adverse

Table 1. Summary of recommendations for viral entry targeted antivirals. A list of the antivirals that target viral entry is provided, as well as the recommended time of treatment during disease progression. Note that only one antiviral is recommended as preventative treatment (before infection has occurred); the majority are recommended for mild to moderate infection, none are recommended for severe infection, and two are not currently authorized for COVID-19 treatment.

Antiviral	Timing of treatment	References
Evusheld	Preventative	[9]
REGEN-COV	Mild to moderate infection	[10]
BNE	Mild to moderate infection	[11,12]
Bebtelovimab	Mild to moderate infection	[13]
Sotrovimab	Mild to moderate infection	[14]
Arbidol	Not authorized for COVID-19 treatment	[15–19]
Chloroquine	Not authorized for COVID-19 treatment	[20]

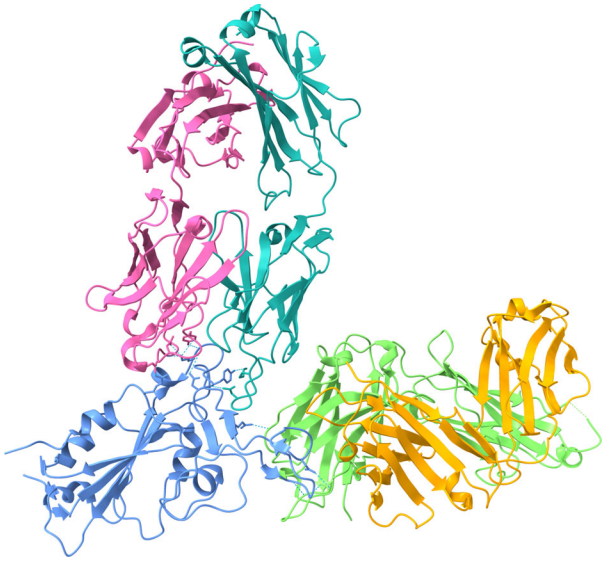

Fig. 2. The component antibodies of Evusheld simultaneously bind to separate regions of the RBD of the SARS-CoV-2 S protein (blue) [Protein Data Bank (PDB) ID: 7L7E, visualized with UCSF CHIMERAX, version 1.4] [21,22]. Tixagevimab consists of a heavy chain (green) and a light chain (orange) that form an aromatic cage. Cilgavimab consists of a heavy chain (pink) that is able to interact with the RBD and a light chain (teal). Hydrogen bonds between each of the antibodies and the RBD are represented by dotted cyan lines.

effects include increased cardiovascular abnormalities, including myocardial infarctions and heart failure; however, no causal relationship has been shown between these effects and Evusheld treatment [9].

The Food and Drug Administration (FDA) then issued an Emergency Use Authorization (EUA) with an authorized dose of 150 mg of each antibody on 16 December 2021, later increasing the approved dose to

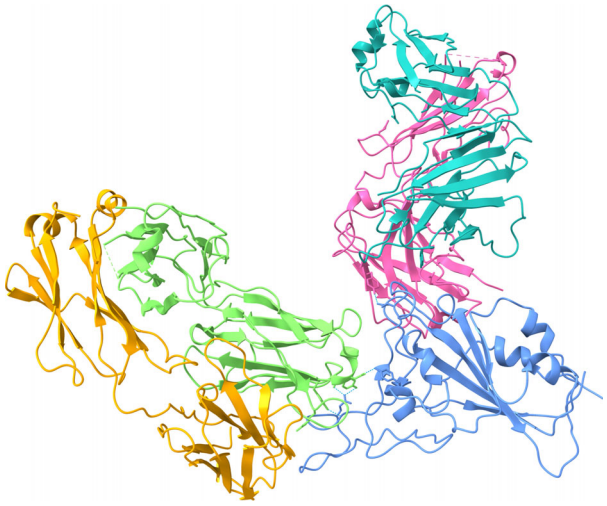

Fig. 3. Casirivimab (REGN 10933, heavy chain: green, light chain: orange) and imdevimab (REGN 10987, heavy chain: pink, light chain: teal) bind mutually exclusive epitopes on a portion of the RBD of the SARS-CoV-2 S protein (blue) (PDB ID: 6XDG, visualized with UCSF CHIMERAX, version 1.4) [31,32]. Casirivimab binds the top of the RBD of the virus and overlaps greatly with the binding site for ACE2. The epitope for imdevimab is located away from the epitope for casirivimab and has little overlap with the ACE2 binding site. The non-overlapping binding sites allow for increased efficiency when binding to the virus due to the multiple binding locations. Hydrogen bonds between each of the antibodies and the RBD are represented by dotted cyan lines.

300 mg on 24 February 2022 [11]. More recently, the European Union also approved Evusheld on 25 March 2022 [26]. Evusheld is only recommended for those who are not infected with COVID-19 at the time of administration and for those unable to receive COVID-19 vaccinations because of allergies to COVID-19 vaccines or a compromised immune system [27,28]. Evusheld is authorized for pre-exposure prophylaxis of COVID-19 infection, even showing reduced viral load for all Omicron variants [29]. Evusheld is not authorized for post-exposure prophylaxis [23,26].

REGEN-COV

REGEN-COV is a IgG1κ monoclonal antibody mixture composed of casirivimab and imdevimab [30]. These antibodies target the RBD of the S protein and prevent viral entry of the SARS-CoV-2 virus into the host cell. The two antibodies work as a combination treatment rather than as an individual monotherapy because they bind to non-overlapping epitopes on the S protein to increase the efficacy of inhibition of viral entry (Fig. 3) [33]. The simultaneous binding to distinct sites of the RBD was intended to protect against

the emergence of new variants of the virus and circulating variants. *In vitro* studies have shown that the cocktail of the two antibodies was effective at presenting the rapid escape of the virus from binding that occurs with single antibodies [34].

On 21 November 2020, the FDA issued an EUA for REGEN-COV to treat mild to moderate COVID-19 cases in adults and children (aged at least 12 years old and weighing 40 kg) who are at high risk of disease progression to severe COVID-19 infection. The treatment is not preventative in nature and is not recommended for treatment of severe COVID-19 infections [10]. The authorized dosage of REGEN-COV is 600 mg of casirivimab and 600 mg of imdevimab administered intravenously in conjunction 10 days after COVID-19 onset. Efforts were made to increase global supply of REGEN-COV with Regeneron's collaboration with Roche, which is primarily responsible for development and distribution outside the USA. The goal was to increase access in developing countries through drug donations to public health organizations [35].

REGEN-COV is still being evaluated as an antiviral treatment for COVID-19. In a previously conducted trial, 799 non-hospitalized adults with mild to moderate COVID-19 symptoms were evaluated. Two hundred and sixty-six patients received 2400 mg of the combination drugs, 267 received 8000 mg of the combination drugs, and 266 received a placebo within 3 days of a positive COVID-19 test. Researchers noted a statistically significant drop of 16.6% in hospitalizations for patients treated with REGEN-COV compared to those receiving a placebo, notably in patients with no previous immune response initiation or with a viral load at baseline measurements [33]. Another study in 2021 showed that REGEN-COV was associated with decreases in hospitalization for any cause or death from any cause, including COVID-19. Eighteen of 1355 patients treated with 2400 mg of REGEN-COV (1.3%) experienced COVID-19-related hospitalization or death, whereas 62 of 1341 patients in the placebo group who underwent randomization (4.6%) experienced such complications. Similarly, such outcomes occurred in seven of 736 patients in the REGEN-COV 1200-mg group (1.0%) and in 24 of 748 patients in the placebo group who underwent randomization. Additionally, five individuals died during the efficacy assessment period, including one in the REGEN-COV 2400-mg group, one in the REGEN-COV 1200-mg group, and three in the placebo group [30]. Other studies have shown that, in cases where one of the antibodies in the cocktail is impacted, the combination treatment retains full neutralization potency of the virus. Furthermore, REGEN-COV is described to retain neutralization capabilities against SARS-CoV-2 variants, including those with S protein mutants with a D614G substitution, the B.1.1.7 variant, and other variants associated with reduced vaccine efficacy (e.g. B.1.351) [34].

Although adverse effects are still being studied, observed effects include symptoms associated with allergic reactions and worsening COVID-19 symptoms after treatment [36,37]. As a result of intravenous administration of the drug, bleeding and bruising of the skin and soreness at the infusion site are also possible.

REGEN-COV is stated to be ineffective against the Omicron variant because of markedly reduced neutralization activity [38]. The specificity of REGEN-COV against the S protein of the virus was reduced as a result of mutations in the virus structure with the rise of Omicron and was stated to be an insufficient treatment. Because of the inability to test for the exact virus variant that each person was infected with, REGEN-COV was limited in its distribution and is no longer authorized for distribution in the US because of the prevalence of Omicron subvariants [39].

Bamlanivimab and etesevimab

Administered as a single intravenous infusion, bamlanivimab and etesevimab (BNE) are two monoclonal antibodies developed by Lilly Investors that, once combined, target the S protein of SARS-CoV-2. The antibodies respectively bind two distinct overlapping sites on the RBD of the S protein, interfering with the ability of the S protein to attach to the ACE2 receptor on the human cell (Fig. 4) [44]. Bamlanivimab was observed to bind to an epitope overlapping the ACE2 binding site: seven of the approximate 25 side chains in the RBD interact with ACE2. This epitope is fully accessible on both the up and down conformations of the binding domain. This property is similar to the binding of the Ebola virus-specific monoclonal antibody 114 that binds the Ebola virus glycoprotein binding domain in both the preactivation and activated states. Etesevimab can only bind to the RBD when it is open, unlike Bamlanvimab [45,46].

Under a 19 February 2021, EUA, the FDA approved BNE for the treatment of mild-to-moderate COVID-19 in children (aged at least 12 years old and weighing 40 kg) and adults at high risk for severe COVID-19 [11,12]. BNE is not authorized for treating patients with severe COVID-19 or underlying COVID-19 comorbidities. The drugs are not approved as a preventative treatment, potentially as a result of an inability to protect against new and upcoming variants

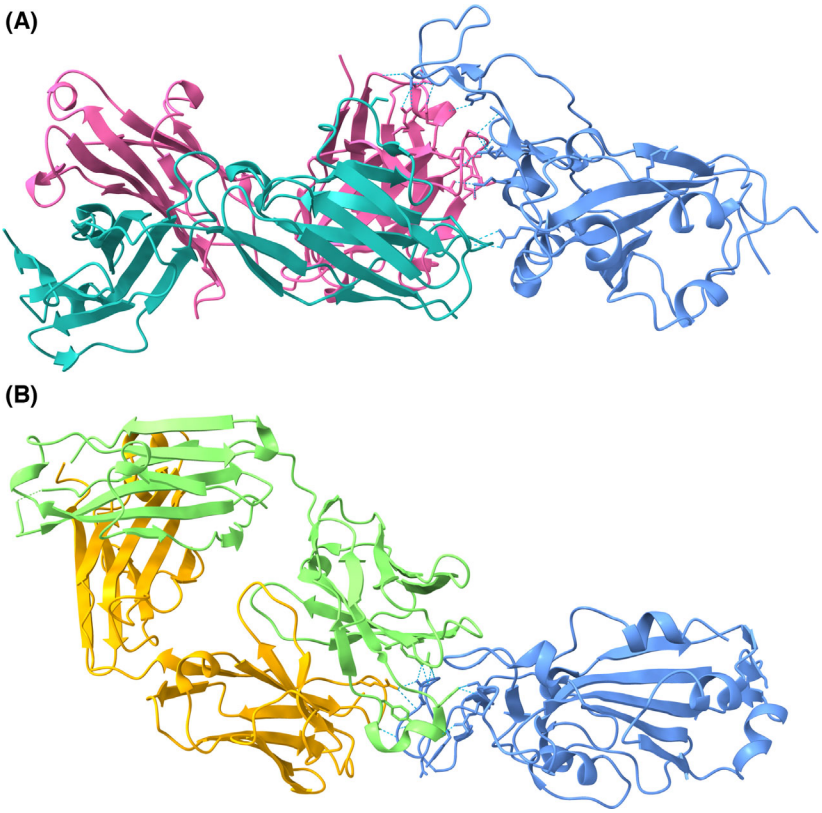

Fig. 4. (A) Etesevimab (heavy chain: pink, light chain: teal) [40] binds to an epitope on a portion of the RBD of the SARS-CoV-2 S protein (blue) overlapping the ACE2 binding site (PDB ID: 7C01, visualized with UCSF CHIMERAX, version 1.4) [41]. (B) Bamlanivimab (heavy chain: green, light chain: orange) [42] binds to an epitope on the RBD that overlaps with the binding site of etesevimab (PDB ID: 7KMG) [43]. Bamlanivimab is able to bind the epitope with the up and down conformations of its monomers [42]. Hydrogen bonds between each of the antibodies and the RBD are represented by dotted cyan lines.

[44]. A procurement agreement allowed multiple European countries to purchase the antibody cocktail directly from Lilly for approved use. The combination package with the cocktail comes with one vial of bamlanivimab (700 mg) and two vials of etesevimab (700 mg) [47].

A phase 2–3 clinical trial was conducted to evaluate drug efficacy and a greater reduction from baseline in the log viral load was observed among patients already testing positive for Covid-19 who received bamlanivimab plus etesevimab compared to those who received placebo. In phase 2, 612 adult patients received a single infusion of the combination drugs of 2800 mg each, an infusion of bamlanivimab alone of 700, 2800 or 7000 mg, and a placebo. On day 11, a 4.8% reduction in hospitalization and death was observed in the group administered the combination drugs compared to the group who received the placebo. In the second trial of the experiment, similar results were obtained [12]. In another study, 1035 patients underwent randomization and received an infusion of bamlanivimab-etesevimab or placebo. By day 29, 11 out of 518 patients (2.1%) in the bamlanivimab-etesevimab group experienced COVID-19-related hospitalization or death from any cause compared with 36 of 517 patients (7.0%) in the placebo group. No deaths occurred in the bamlanivimab-etesevimab group, whereas 10 occurred in the placebo group.

Adverse effects of the drug include hypersensitivity reactions, anaphylaxis, and the possible worsening of COVID-19 symptoms. Worsening symptoms may be a result of the progression of COVID-19 or the combination treatment, and neither has been ruled out as a potential cause. Other possible adverse effects associated with intravenous administration include bleeding, skin bruising, soreness, swelling, and infection at the infusion site [44].

Similar to REGEN-COV, the efficacy of BNE is noted to be markedly reduced with the Omicron variant because of increased resistance of the virus as a result of changes in the S protein and binding domains. The specificity of the binding capabilities of BNE is thus reduced against such variants and it is considered to be an insufficient treatment. BNE was minimally distributed during the height of the Omicron pandemic for this reason and is authorized for distribution in the USA in most states and territories [39]. It has remained effective against other global variants.

Bebtelovimab

Developed by Eli Lilly, bebtelovimab (also known as LY-CoV1404) is a human IgG1 monoclonal antibody that targets the RBD of the SARS-CoV-2 S protein, preventing the virus from binding with ACE2 to trigger endocytic entry into the cell (Fig. 5). Both the up and down conformers of the RBD on the SARS-CoV-2 S protein can be bound by the fragment antigen-binding domain of the antibody. The epitope, being exposed in both conformations, allows bebtelovimab to bind to the S protein regardless of conformation, which is not a feature of many other monoclonal antibodies targeting the RBD. Multiple molecules of bebtelovimab can bind to the 3-RBD-up conformation of the S protein (which has all three RBDs on the trimeric S protein exposed for binding to the ACE2 receptors), although only one molecule of bebtelovimab can bind to the 3-RBD-down conformation (in which the S protein cannot bind to ACE2, on account of the RBDs being partially buried). Binding affinities were determined to be relatively high for bebtelovimab binding to the S protein, ranging from a binding constant (K_D) of 75 to 220 pM. Furthermore, *in vitro* viral neutralization studies revealed bebtelovimab to have a higher potency than the comparable monoclonal antibody bamlanivimab because the half maximal inhibitory concentration (IC_{50}) of bebtelovimab was consistently two to three times lower than that of bamlanivimab [48,50].

Bebtelovimab was authorized for use in the treatment of mild-to-moderate COVID-19 infection on 11 February 2022, by the FDA under an EUA [13]. Bebtelovimab is able to bind and neutralize all current variants of concern as of June 2022, including the Omicron variant and its subvariants (BA.1, BA.2, BA.4, and BA.5). No other monoclonal antibody has been shown to be effective against all variants of concern, making bebtelovimab a promising therapeutic [48,50–52].

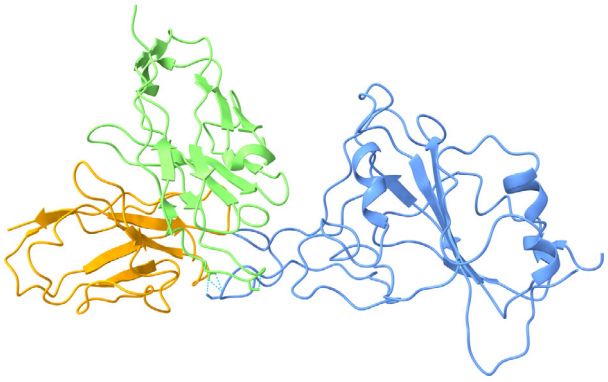

Fig. 5. Bebtelovimab interacting with a component of the RBD of the SARS-CoV-2 S protein (PDB ID: 7MMO, visualized with UCSF CHIMERAX, version 1.4) [48,49]. The S protein is blue, the light chain of bebtelovimab is orange, and the heavy chain of bebtelovimab is green. Hydrogen bonds between bebtelovimab and the RBD are represented by dotted cyan lines.

The population intended for treatment with bebtelovimab under the FDA's EUA is adults and children (aged at least 12 years old and weighing 40 kg) who test positive for COVID-19 and are at high risk for progression to severe COVID-19. Bebtelovimab is administered via a single 175-mg intravenous injection, providing an advantage over therapeutics administered over multiple days [13]. As of July 2022, bebtelovimab remains only authorized for use in the USA and no applications for authorization or approval have been submitted in any other country [53].

A phase 2 clinical trial tested the ability of bebtelovimab individually and in conjunction with BNE to reduce the proportion of patients with persistently high viral load from baseline to day 11. Bebtelovimab administered with BNE in low-risk patients produced a 36% relative risk reduction, whereas bebtelovimab administered on its own in low-risk patients produced a 40% relative risk reduction and reduced the time for sustained symptom resolution by a median of 2 days compared to placebo; these results were statistically significant at a level of $\alpha = 0.01$ or below [54]. Nausea and vomiting were the most common adverse effects observed in patients who received bebtelovimab, alone or in conjunction with BNE, at 0.8% and 0.7%, respectively [55].

The epitope on the S protein targeted by bebtelovimab is notably more conserved than epitopes targeted by other monoclonal antibodies. This fact, in conjunction with its ability to tolerate mutations in the S protein at the less conserved N439 and N501 sites, explains its ability to effectively inhibit all current variants of concern; N439K and N501Y mutations that have been observed in current SARS-CoV-2 strains have no effect on bebtelovimab binding or neutralization efficiency. However, individual substitution mutations at positions 444 (K444Q), 445 (V445A), and 499 (P499R, P449S) did lead to a notable decrease in ACE2 competition, binding, and neutralization activity, although these mutations are not common in the global population of SARS-CoV-2 strains; resistance to bebtelovimab may evolve if any of these mutations occur in a future variant of concern [48].

Sotrovimab

Developed by GlaxoSmithKline and Vir Biotechnology, sotrovimab is a monoclonal antibody that was

developed from the antibody S309 (Fig. 6). S309 was originally isolated from the memory B cells of a patient who recovered from severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 [58]. This was carried out to demonstrate how the epitope of the antigen was assumed to be highly conserved.

Sotrovimab is a IgG1κ monoclonal antibody composed of two identical light chain polypeptides (214 amino acids) and two identical heavy chain polypeptides (457 amino acids). The drug binds to a conservative epitope on the S protein RBD of SARS-CoV-2. Sotrovimab inhibits an unknown step between virus attachment and the fusion of viral and cell membranes [59]. Further work is needed to elucidate the mechanism of inhibition.

Sotrovimab was approved under a September 2021 EUA for mild-to-moderate COVID-19 cases in adults and children (aged at least 12 years old and weighing 40 kg), but it is currently not authorized for use in the US due to Omicron prevalence. The drug should be administered within 7 days to COVID-19-positive individuals who do not require hospitalization or respiratory support. It is given in the form of 500 mg of solution diluted via intravenous diffusion over the course of 30 min, followed by a monitoring period of 1 h [14].

In the COMET-ICE trial, 1057 subjects were given a sotrovimab or placebo infusion. After 29 days, the adjusted relative risk reduction was 79% compared to the placebo [60]. In a similar clinical trial called COMET-TAIL, 385 subjects received the sotrovimab infusion. After 29 days, only 1.3% of patients had a progression that led to hospitalization over 24 h. Adverse effects of this treatment include anaphylaxis or infusion-related reactions such as fevers, difficulty breathing, arrhythmia, fatigue, and chest pain [61].

However, because sotrovimab only targets one viral epitope, this means that mutations can lead to resistance to the drug, as shown by a study using patients from the Western Sydney Local Health District in New South Wales, Australia. Analyzing the first 100 patients who received treatment of sotrovimab at the facility, it was discovered that eight patients had remained positive for the virus when PCR testing was performed. The genomic analysis then confirmed that mutations occurred in four of the eight patients that had led to viral resistance against the drug [62].

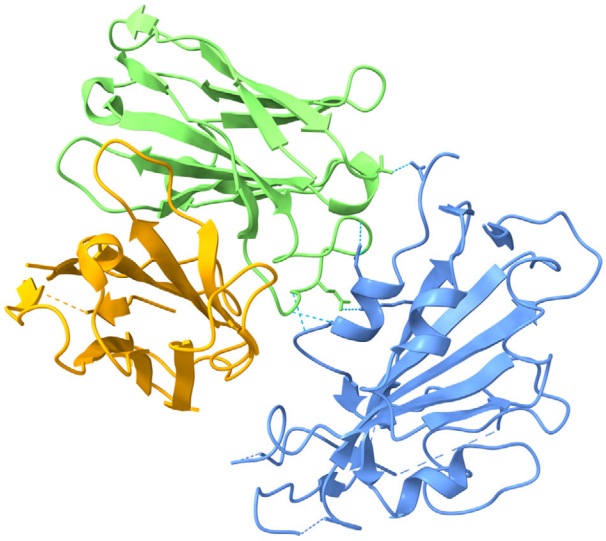

Fig. 6. S309, the parent antibody of Sotrovimab, interacting with a portion of the RBD of the SARS-CoV-2 S protein (PDB ID: 6WPS, visualized with UCSF CHIMERAX, version 1.4) [56,57]. S309 consists of a heavy chain (green) and a light chain (orange). Hydrogen bonds between S309 and the RBD are represented by dotted cyan lines.

Arbidol

Umifenovir, brand-name Arbidol, is a hydrophobic, indole-derived antiviral agent manufactured by Russian pharmaceutical company OTCPharm [63]. Arbidol has been used for approximately 25 years in Russia and for 16 years in China as a prophylactic treatment for influenza and other respiratory infections [64], but has not been approved anywhere for use against COVID-19. It has been well established that Arbidol acts to inhibit viral fusion with the host-cell membrane by binding to the virus and the membrane simultaneously, thus preventing viral endocytosis [63,65,66].

Because of the success of Arbidol against a wide array of viral pathogens, the drug has been investigated as a therapeutic agent against SARS-CoV-2. Much like in influenza, Arbidol binds dually to the RBD of the SARS-CoV-2 S protein and ACE2 receptors on the host-cell membrane, forming stronger interactions with the RBD than ACE2 (Fig. 7) [67,68]. This higher binding affinity of Arbidol to the RBD is the result of multiple residues including Arg403, Asp405, Glu406, Gln409, Gly416, Lys417, Ile418, and Tyr505 [67]. Arbidol is proposed to interact with three identical binding sites on each of the three subunits of the S protein trimer [67–69]. The triple binding in this region is suggested to prevent trimerization of the S protein, a necessary step in viral entry [68]. Specifically, Arbidol increases the affinity of the RBD to ACE2, but induces rigidity in the RBD-ACE2 complex, preventing it from forming the conformation necessary for the assembly of endosomes [67]. This interaction is sufficient for inhibition of viral entry

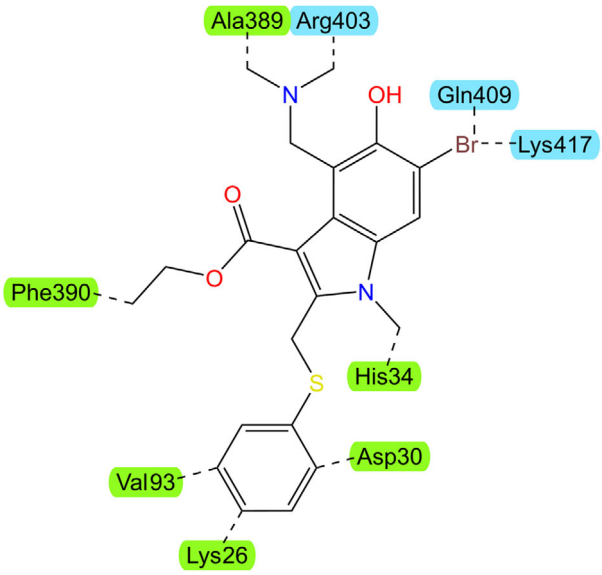

Fig. 7. The chemical structure of Arbidol, an indolic carboxylic acid. Dashed lines indicate interactions between dually-bound Arbidol and residues on ACE2 (green label) and the RBD of the SARS-CoV-2 S protein (light blue label). The ability of Arbidol to bind dually with the RBD and ACE2 is assumed to prevent the S-protein from forming the necessary configuration for viral uptake into the host-cell. Adapted from Padhi *et al.* [67].

[69]. Additionally, an *in vitro* study has also shown that Arbidol may also impede release of the virus from intracellular vesicles [70], suggesting that the drug may additionally disrupt S-activation impeding an interaction between the S protein and cathepsin L in the endolysosome. However, studies must be performed to test this hypothesis and elucidate the mechanism by which Arbidol inhibits the release of SARS-CoV-2 from intracellular vesicles.

In vitro studies established Arbidol as having very promising antiviral effects on SARS-CoV-2, demonstrating statistically significant decreases in binding efficiency, inhibition of viral entry, and inhibition of post-viral events [70]. However, clinical trials have been inconclusive. One study of 100 individuals with COVID-19 in a teaching hospital found that Arbidol significantly contributed to clinical and laboratory improvements, including peripheral oxygen saturation, hospitalization duration, white blood-cell count, erythrocyte sedimentation rate, intensive care unit admissions, and chest computed tomography involvements [71]. However, a study of 81 COVID-19 patients in a non-intensive care unit ward, as well as another study of 101 moderate-severe COVID-19, found no significant difference in SARS-CoV-2 clearance or prognosis [15,16]. Another study shows that the use of Arbidol as a preventative measure led to significantly decreased infection rates in health professionals (N = 164), although the same study reports no significant difference in hospitalization rate in infected individuals [17]. Furthermore, one study even reported increased in-hospital mortality in severe COVID-19 patients when Arbidol was administered alone or in combination with Oseltamivir [18]. This study used a total of 109 patients with 23 receiving Arbidol monotherapy, 10 receiving the Arbidol and Oseltamivir combination, and the remainder receiving other antiviral therapeutics, none of which resulted in the same increased in-hospital mortality [18]. The discrepancy between Arbidol efficacy *in vitro* versus clinical trials could be a result of the dosage administered to patients [15]. *In vitro* studies utilize high dosages of the drug to obtain the inhibitory effects but, because of concerns with side effects, high doses of Arbidol cannot be prescribed to patients [15,19]. The efficacy of Arbidol remains controversial and thus more studies are needed to show its safety and efficacy *in vivo*.

Arbidol is orally administered three times daily in doses of 200 mg [15]. Additionally, Arbidol has been shown to have mild adverse effects, including nausea, diarrhea, dizziness, and elevated serum transaminase, and it should be used cautiously in patients with liver dysfunction [72]. No data have been reported regarding the development of Arbidol resistance in SARS-CoV-2. One study exploring new potential antiviral compounds reports a binding affinity of Arbidol to the Omicron strain S protein as -20.88 kJ·mol^{-1}, which is weaker than the reported binding affinity of Arbidol to the 2019 strain of -105.95 ± 14.25 kJ·mol^{-1}, indicating a weaker interaction with the newer strain [73]. However, the reasons for this reduced affinity, as well as the consequences, have not been reported.

Endosomal targeting

Chloroquine

Originally introduced as an antimalarial drug and used as an antiviral since the 1960s, chloroquine is now being used to treat autoimmune diseases such as lupus and rheumatoid arthritis in clinical trials (Fig. 8). Chloroquine is continually aiding the global healthcare system by being widely accessible and low-cost [74].

Chloroquine alters the pH of endosomes/lysosomes, creating an alkaline environment that prevents binding and endocytosis *in vitro* [8]. Additionally, chloroquine disrupts the glycosylation of the cellular receptors of severe acute respiratory syndrome coronavirus (SARS-CoV) [75]. Specifically, the number of Toll-like

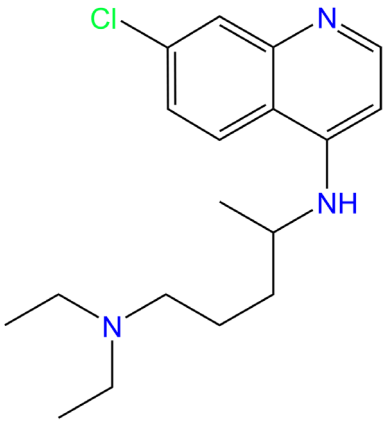

Fig. 8. Chemical structure of chloroquine, an aminoquinoline that increases the pH of endosomes. Chloroquine has been used to treat malaria, viral diseases, and autoimmune diseases and was investigated for use against SARS-CoV-2 by preventing endosomal entry in the early stages of the pandemic [8,74].

receptor signaling molecules is reduced, which eventually lowers cellular cytokine levels. The lowering of cytokine levels eventually leads to the blocking of viral activity within the cell. This reduction mitigates the cytokine storm that develops in the cell as a result of COVID-19 [75]. Because of its *in vitro* success with SARS-CoV, chloroquine was investigated for use against SARS-CoV-2 [20].

In March 2020, the FDA issued an EUA to administer chloroquine for severe cases of COVID-19 [74]. However, the use of chloroquine for COVID-19 has spurred controversy because of a lack of well-designed clinical studies supporting the efficacy of chloroquine in treating COVID-19. There are clinical trials testing the efficacy of chloroquine (23 trials in China alone), yet none have produced sufficient evidence of the benefits of chloroquine for COVID-19 [20]. The WHO views chloroquine use for COVID-19 as solely experimental, and so it must be designated as off-label to be prescribed. Chloroquine also has a narrow margin between beneficial and toxic doses. For short periods of use, the adverse effects of chloroquine are considered mild, with mainly gastrointestinal reactions, dizziness, and headaches. The most severe adverse effects from short-term use can be seizures or psychosis, although these quickly dissipate after treatment [74].

Long-term use of chloroquine has been linked to serious adverse effects. Over time, the toxicity of chloroquine can lead to retinopathy, circular defects, and retinal diameter defects. These are permanent conditions that are caused by the accumulation of chloroquine in the eyes. Chloroquine poisoning is also linked to cardiovascular disorders. Overall, the grave adverse effects that arise from long-term use have called into question the benefits of this drug as a therapeutic for COVID-19 [20].

Table 2. Summary of recommendations for viral replication targeted antivirals. A list of all of the antivirals discussed in the viral replication section is provided, as well as an indication of when in the progression of the disease they are recommended. Note that no antivirals are recommended as preventative treatment (before one is infected); the majority are recommended for mild to moderate infection, only one is recommended for individuals who might develop severe infection, and three are not currently authorized for COVID-19 treatment.

Antiviral	Timing of treatment	References
Paxlovid	Mild to moderate infection	[76,77]
Kaletra	Not authorized for COVID-19 treatment	[78]
Remdesivir	Infection with high risk to develop severe infection	[79]
Ribavirin	Not authorized for COVID-19 treatment	[48,49]
Favipiravir	Not authorized for COVID-19 treatment	[80]
Molnupiravir	Mild to moderate infection	[81]

Viral replication

The treatments in this section target the maturation process of the virus once it is already in a cell. Here, we focus on treatments targeting protease inhibition, RdRp stalling, and error catastrophe. A summary of these treatments and their timing is found in Table 2.

Protease inhibition

The main protease of SARS-CoV-2 (M^{pro}) is a 3-chymotrypsin-like cysteine protease ($3CL^{pro}$). Following the translation of viral mRNA by ribosomes in the host cell, M^{pro} produces non-structural proteins (NSPs) by cleaving peptide bonds in 11 distinct sites on the two viral polyproteins (polyproteins 1a and 1ab), which are coded for by overlapping sections in open reading frame 1ab of the viral genome. The catalytic dyad of the His41 and Cys145 residues form the catalytic site of M^{pro}. Additionally, the substrate binding sites of M^{pro} are highly conserved among coronaviruses. NSP5-NSP16 are produced by the actions of M^{pro} and NSP4 is produced by the combined actions of M^{pro} (which is NSP5) and the papain-like protease (NSP3) of SARS-CoV-2 (which is also responsible for cleaving these polyproteins to produce NSP1-NSP3). Many of these NSPs are involved in the formation of the replication-transcription complex of SARS-CoV-2;

for example, NSP12 is RdRp, with NSP7 and NSP8 being cofactors of RdRp, whereas NSP13 is a helicase and nucleoside-triphosphatase. Anchored in place by NSP3, NSP4, and NSP6, the replication-transcription complexes are localized to convoluted membrane structures in the rough endoplasmic reticulum. Therefore, inhibiting M^{pro} impedes viral replication because the necessary proteins that form the replication-transcription complex will not be produced, making it a promising candidate for targeting by therapeutics. The downstream targeting of RdRp, a product produced by the enzymatic activity of M^{pro}, is also be discussed later in this section [82–88].

Paxlovid

Paxlovid, developed specifically in response to the COVID-19 pandemic by Pfizer, is a combination treatment of nirmatrelvir (also known as PF-07321332) and ritonavir (Fig. 9A,B) [90,91].

Ritonavir is a pharmacokinetic enhancer that increases the half-life of nirmatrelvir and other pharmaceuticals by inhibiting cytochrome P450 3A4, a major enzyme involved in human drug metabolism. The N-terminus thiazole group of ritonavir irreversibly binds to heme iron within the cytochrome P450 3A4 active site to inhibit the enzyme [92,93]. Because ritonavir inhibits drug metabolism by cytochrome P450 3A4, Paxlovid is not advised to be administered in tandem with pharmaceuticals relying on this enzyme, which limits the population that can benefit from treatment with Paxlovid [76]. Ritonavir also induces other cytochrome P isoenzymes and uridine diphosphate glucuronosyltransferases, a class of drug-metabolizing enzymes, at the same time as inhibiting the efflux transporter P-glycoprotein and organic anion transporting polypeptides 1B1 and 1B3 [93].

Nirmatrelvir inhibits the main protease (M^{pro}) of SARS-CoV-2, which is a 3-chymotrypsin-like cysteine protease [90,94–96]. When not inhibited, M^{pro}

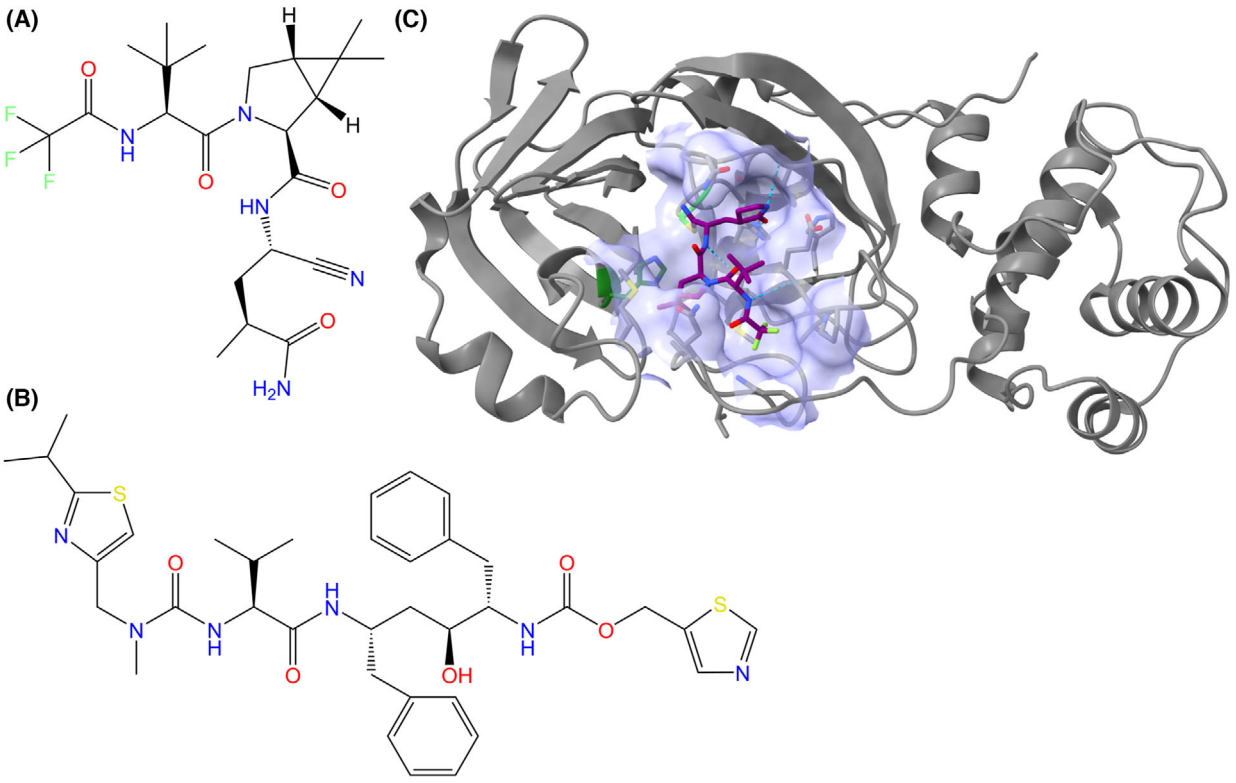

Fig. 9. Chemical structures of (A) nirmatrelvir and (B) ritonavir, the two active components of Paxlovid. (C) Nirmatrelvir in complex with the M^{pro} of SARS-CoV-2 (PDB ID: 7SI9, visualized with UCSF CHIMERAX, version 1.4) [89], with the binding pocket (lavender, composed of atoms within 5 Å of the inhibitor) visualized [82]. Nirmatrelvir is represented by a stick model with a purple carbon backbone. The peptide backbone of M^{pro} is represented in gray, with secondary structures displayed in cartoon format. In the binding pocket, stick models of amino acid residues are shown, and the catalytic dyad of M^{pro} (His41 and Cys145) is emphasized with carbon atoms in green. Hydrogen bonds between nirmatrelvir and M^{pro} are represented by dotted cyan lines, and heteroatom colors correspond to those in the 2D visualization.

proteolytically processes proteins that are critical for viral replication, including RdRp and other instances of M^{pro} [94]. Nirmatrelvir inhibits M^{pro} via reversible covalent bonding between the nitrile warhead of nirmatrelvir and the catalytic cysteine (Cys145) of M^{pro}, which causes a thioimidate group to form (Fig. 9C). Prior to this occurring, nirmatrelvir forms a non-covalent complex with M^{pro} [95,96].

Computational models suggest that the non-covalent complex between M^{pro} and nirmatrelvir and the subsequent steps for covalent bond formation occur via the following interactions. The P1 γ-lactam ring of nirmatrelvir mimics interactions of the enzyme with glutamine, which occurs before cleavage sites in the SARS-CoV-2 polyproteins. The bicycloproline component of nirmatrelvir also mimics a leucine residue, allowing it to fit into the S2 site of M^{pro}, and it has a stacking interaction with the catalytic His41. The P3 position of nirmatrelvir contains a *tert*-butyl group that maintains similar interactions to valine residues at cleavage sites on the polyproteins, and nitrogen and oxygen atoms in this position hydrogen bond with Glu166, which plays a major role in the binding of both the intended substrate peptides and nirmatrelvir. P4 has the largest difference between nirmatrelvir and the serine residue with respect to peptide substrate interactions with the enzyme because the fluorine atoms in nirmatrelvir form hydrogen bonds with Glu192 opposed to Glu166, Gln189, and Thr190 for the serine residue in the peptide substrate. The formation of the covalent bond between nirmatrelvir and M^{pro} results in the electrophilic carbon atom of nirmatrelvir's nitrile group being attacked by a nucleophilic thiol group on the cysteine residue, which is first activated via proton transfer to the catalytic histidine (His41). Once the nucleophilic attack has taken place, a second proton transfer from His41 to nirmatrelvir occurs to complete the reaction [95,96].

On 22 December 2021, the FDA issued an EUA for the use of Paxlovid in the treatment of COVID-19 in adults and children (aged at least 12 years old and weighing 40 kg) who have tested positively for SARS-CoV-2 and display mild-to-moderate symptoms with a high risk of advancing to severe COVID-19 symptoms. Paxlovid is not authorized for preventative use by the FDA [76,77]. Standard treatment protocols for Paxlovid call for 300 mg of nirmatrelvir and 100 mg of ritonavir to be administered orally, twice daily for five consecutive days [77,90].

Paxlovid has also been authorized for use in the treatment of COVID-19 across the European Union and other countries such as the UK, Israel, and China. The WHO currently recommends the use of Paxlovid in COVID-19 patients a high risk for hospitalization, and Pfizer has entered a Medicines Patent Pool agreement with the WHO to allow for the production of generic therapeutics that are bioequivalent to Paxlovid in 95 low- and middle-income countries [97,98].

In the EPIC-HR phase 2–3 clinical trial, Paxlovid treatment within 5 days of symptom onset reduced COVID-19 related hospitalizations and death by 89.1% among patients who had not received monoclonal antibody treatments for COVID-19 compared to placebo [77,90]. In this trial, 7.8% of patients treated with Paxlovid experienced one or more adverse effects, only two of which affected over 1% of patients treated with Paxlovid: 4.5% experienced an impaired sense of taste (dysgeusia) and 1.3% experienced diarrhea [90].

Pfizer recently announced early findings from the Phase 2/3 EPIC-PEP clinical trial that analyzed the post-exposure prophylactic potential of Paxlovid; against placebo, the trial found a 32% and 37% risk reduction in adults who took Paxlovid for 5 and 10 days, respectively, after being exposed to a symptomatic household contact who tested positive for SARS-CoV-2, but these results lacked statistical significance. Additionally, in the Phase 2/3 EPIC-SR trial, a lack of statistically significant findings in risk reduction for standard risk patients and a low observed risk of hospitalization and death caused Pfizer to cease enrollment, affirming the decision by the FDA to limit the use of Paxlovid under the current EUA to only patients who are at a high risk of progression to severe COVID-19 symptoms. Full data and results from the EPIC-PEP and EPIC-SR trials will be published in the coming months [99–102].

Additionally, cases of 'Paxlovid rebound' have recently been reported in the media and literature, with mild COVID-19 symptoms recurring after initial recovery post-treatment with Paxlovid; early research suggests that insufficient drug exposure is a likely cause of this phenomenon because infected cells not exposed to the drug would allow for continued viral replication and a reemergence of symptoms [103–105]. However, these rebound cases remain rare because, in the Paxlovid clinical trial, < 1% of 5287 patients were hospitalized (six patients) or required care in an emergency department for COVID-19 symptoms (39 patients) 5–15 days after treatment with Paxlovid [103,106].

Nirmatrelvir has been found to have a similar level of efficacy across variants with missense mutations affecting M^{pro}, including C.37 (Lambda), B.1.1.318, B.1.2, B.1.351 (Beta), B.1.1.529 (Omicron), and P.2 (Zeta). However, none of the M^{pro} missense mutations

in the current variants take place at the active site of the enzyme, meaning that there are still many questions about how such a mutation would affect the inhibition potential of nirmatrelvir [107,108]. Because nirmatrelvir shares many of the same interactions with M^{pro} that the peptide substrate uses, mutations that prevent nirmatrelvir from inhibiting M^{pro} will likely also decrease the binding efficiency of the substrate; however, the low conservation of the flexible amino acid 45–51 region may allow for the evolution of resistance, and mutations in other regions that directly interact with nirmatrelvir, such as 163–169 and 186–192, may also impart resistance [109].

Indeed, resistance to nirmatrelvir has been reported for individual M^{pro} mutants with at least one of the following amino acid substitutions, many of which occur in the aforementioned regions: L50F, S144M/F/A/G/Y, M165T, E166A/Q/V, L167F, H172Q/F, and Q192T/S/V. Computationally, an M^{pro} mutant with two substitutions, L50F and E166V, conferred up to an 80-fold resistance to nirmatrelvir in homologous infectious cell culture and, *in vitro*, a triple mutant (L50F, E166A, and L167F) was reported to have a 72-fold increase in resistance compared to wild-type M^{pro}, although at the sacrifice of only having 5.3% of the enzymatic activity of the wild-type [110–112]. This finding implies that mutations in the active site of M^{pro}, although imparting nirmatrelvir resistance, may reduce the overall fitness of the virus because of the many shared protein–inhibitor and protein–substrate interactions, potentially reducing its capacity for infection. However, because M^{pro} has a highly conserved structure and a significantly lower mutation rate than other SARS-CoV-2 proteins (such as the S protein), resistance to nirmatrelvir may be slow to evolve for variants in the wild [109,113].

Kaletra

Kaletra is a combination treatment of lopinavir and ritonavir (Fig. 10), originally developed by Abbott Laboratories to treat HIV-1 infections [115–117]. It was initially approved for use in this manner by the FDA in September of 2000 [118]. Lopinavir inhibits the $3CL^{pro}$ of HIV by targeting the C2-symmetric pocket of the active site. Ritonavir, as in Paxlovid, acts as a pharmacokinetic enhancer to increase lopinavir half-life [119]. Because lopinavir–ritonavir was also proposed to inhibit the $3CL^{pro}$ of SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) based on the results of *in vitro*, *in vivo*, computational, and clinical studies, it was logical to test its efficacy against SARS-CoV-2 in the initial

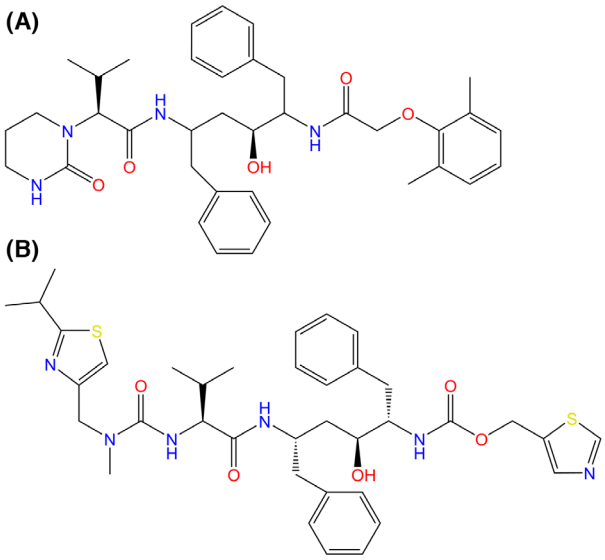

Fig. 10. Chemical structures of (A) lopinavir and (B) ritonavir, the two components of Kaletra. Kaletra was originally developed to treat HIV-1 infections and its potency in fighting SARS-CoV-2 infections was investigated during the early stages of the COVID-19 pandemic as a potential protease inhibitor [114].

stages of the pandemic despite a lack of evidence for a specific mechanism of inhibition of the prior coronaviruses [114,120].

Although an *in vitro* study did find lopinavir to have a 50% effective concentration of 26.63 μM against SARS-CoV-2 in Vero E6 cells [115], SARS-CoV-2 M^{pro} and other coronavirus proteases (which are also $3CL^{pro}$s) do not contain the C2-symmetric pocket, and so questions remain about a potential mechanism for antiviral action against SARS-CoV-2 [119].

Conversely, a randomized control trial of 199 patients in China reported no significant difference in recovery time, 28-day mortality or viral load for lopinavir–ritonavir treatment compared to the control [121]. Another study obtained similar results with a larger sample size of 1616 patients in the UK, finding that treatment with lopinavir–ritonavir was not associated with a reduced risk of progressing to mechanical ventilation or death, 28-day mortality, or hospital stay duration [122].

Diarrhea was a common adverse drug reaction for patients taking lopinavir–ritonavir, even outside of its use in the early stages of the COVID-19 pandemic, because approximately 50% of patients in clinical trials for HIV were reported to experience this adverse event; a retrospective study in one hospital found this combination treatment to be the therapy most implicated in the occurrence of adverse effects among those

initially prescribed in the early days of the COVID-19 pandemic, outclassing even hydroxychloroquine [123].

Given the lack of evidence for the efficacy of lopinavir as an antiviral therapeutic for SARS-CoV-2, further large-scale clinical trials of lopinavir–ritonavir have not been pursued at this time and the National Institutes of Health (NIH) COVID-19 Treatment Guidelines Panel strongly recommends against the use of lopinavir–ritonavir in the treatment of SARS-CoV-2 infection [78].

RNA-dependent RNA polymerase stalling

A class of COVID-19 drugs that have proven to be effective in recent clinical trials comprises the RdRp inhibitors. RdRp enzymes are instrumental in the replication and transcription mechanisms of coronaviruses such as COVID-19. In SARS-CoV-2 specifically, the RdRp complex is composed of an NSP12 core catalytic subunit, an NSP7–NSP8 heterodimer, and another NSP8 subunit. These components work in conjunction to allow incoming nucleoside triphosphate (NTP) substrates bind to the +1 site, whereas the 3′-terminal nucleotide of the RNA product remains in the −1 site. Additional ribonucleotides are incorporated via the catalytic cycle, thereby growing the RNA product strand [124].

Therapeutics such as remdesivir, ribavirin, and favipiravir act as nucleoside analogs: they compete with nucleosides to form strong hydrogen bonds with mRNA, thus disrupting RNA replication via a stalling mechanism [125].

Remdesivir

Remdesivir is a phosphoramidate prodrug that produces the active NTP analog remdesivir triphosphate (RTP) when metabolized. When RdRp uses RTP as a substrate, remdesivir monophosphate (RMP) is incorporated into the RNA product strand (Fig. 11). As a result of the function of remdesivir as an adenosine analog, RMP is able to base pair with uridine

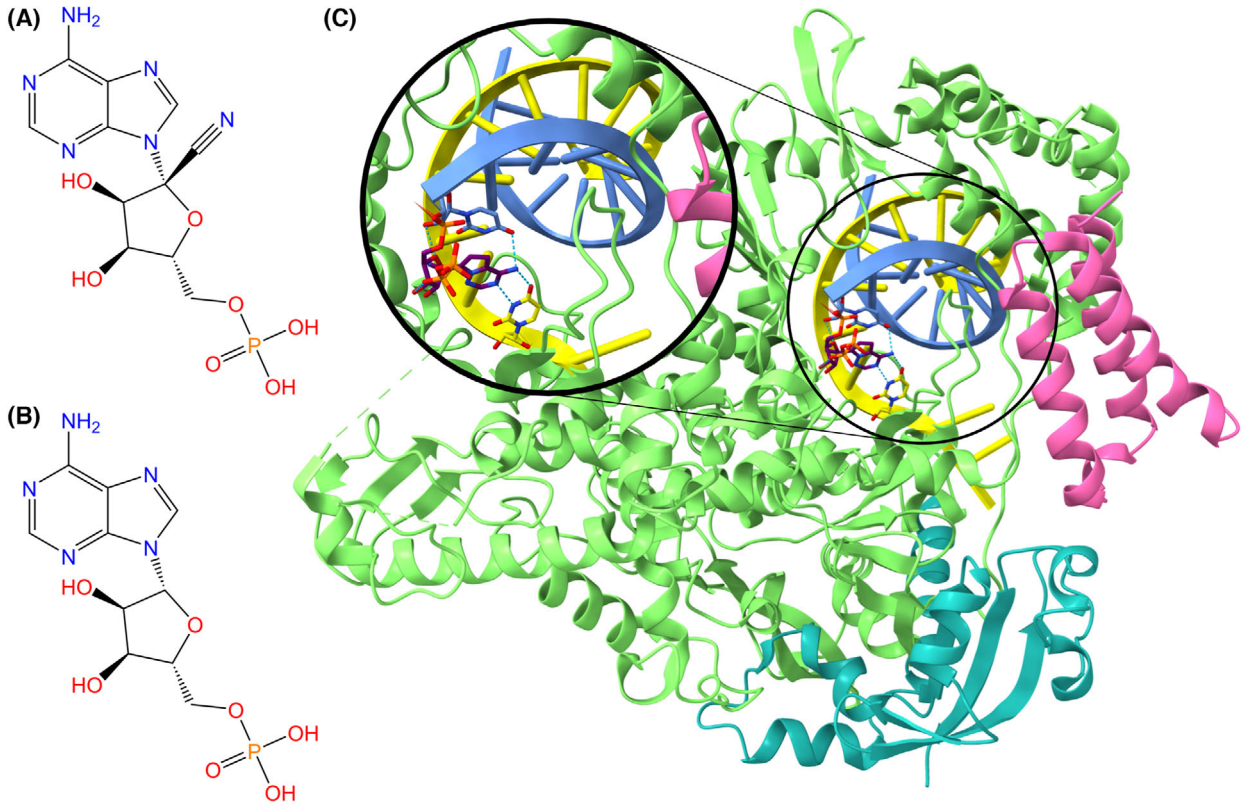

Fig. 11. (A) Structures of remdesivir monophosphate and (B) AMP. Remdesivir functions as an adenosine analog, such that RMP can replace AMP in the product RNA, thereby inhibiting the RdRp viral replication mechanism. (C) RdRp complex (NSP12: green, NSP7: pink, NSP8: teal) bound to template (yellow) and primer (blue) RNA strands and remdesivir triphosphate (purple backbone) (PDB ID: 7BV2, visualized with UCSF CHIMERAX, version 1.4) [124,126]. Hydrogen bonds between remdesivir triphosphate and RdRp or the RNA strands are represented by dotted cyan lines, and heteroatom colors correspond to those in the 2D visualization.

monophosphate and thus replace AMP in the product RNA. Kokic *et al*. [127] found that RdRp was able to overcome the stalling mechanism of remdesivir at higher concentrations of NTP, whereas RdRp inhibition was strong with higher concentrations of RMP.

The RdRp stalling mechanism is specific to SARS-CoV-2 in that RMP incorporation by the RdRp results in a three nucleotide extension of the product strand, as opposed to the five nucleotide extension seen in Ebola virus [128]. This limit between position –3 and –4 is known as the translocation barrier, meaning that additional RMPs cannot be incorporated into the RNA product past position –3. The translocation barrier is caused by steric hindrance at position –4 between the C1′-cyano group in the ribose part of remdesivir and a S861 side chain in NSP12.

Stalling the RdRp replication and transcription mechanism in turn inhibits the proliferation of SARS-CoV-2 *in vitro* and *in vivo*. This impaired growth eventually leads to degradation of the viral RNA genome [127].

On 22 October 2020, remdesivir (known commercially as Veklury) became the first drug approved by the FDA for COVID-19 treatment, in patients aged at least 12 years and weighing 40 kg. On 21 January 2022, its use was expanded to include COVID-19 treatment in non-hospitalized patients with positive results of direct viral testing who are at high risk for progression to severe COVID-19 [79]. On 25 April 2022, remdesivir was additionally approved for COVID-19 treatment in hospitalized or high-risk pediatric patients aged at least 28 days and weighing 3.0 kg by the FDA under a supplemental new drug application (replacing the EUA), making it the first approved treatment for children under 12 years of age [129].

The original decision by the FDA to approve remdesivir for use on hospitalized patients was supported by a randomized, placebo-controlled trial in which 1062 hospitalized patients with moderate-to-severe COVID-19 received either remdesivir or a placebo. The dosing procedure involved 200 mg of remdesivir as a loading dose on day 1, followed by 100-mg doses each day for as many as additional 9 days or until recovery. The patients who received remdesivir had a median 10-day recovery period and a 6.7% mortality rate compared to a 15-day recovery period and 11.9% mortality rate for those who received the placebo [130]. The decision to expand remdesivir for use in non-hospitalized COVID-19 patients was informed by a randomized trial of 562 patients that showed an 87% reduction in risk of hospitalization or death among high-risk, non-hospitalized patients who received a 3-day course of remdesivir compared to those who received the placebo [131].

In a press release dated 25 June 2020, announced by the European Medicines Agency, remdesivir became the first medicine recommended for authorization in the European Union, and later became the first antiviral fully licensed for COVID-19 treatment in Europe [132,133]. The European Commission expanded remdesivir approval on 21 December 2021 to include adults who do not require supplemental oxygen but are still at a high risk of progressing to severe COVID-19. However, recent trials undertaken by Gilead and WHO involving patients hospitalized for COVID-19 showed no significant difference in symptom improvement between those who received remdesivir and those who received no study drug [134,135].

In March 2022, the first remdesivir-resistant variant of SARS-CoV-2 was identified in an immunocompromised patient. The resistance arose as a result of a mutation, E802D, in NSP12. Similar resistance has been demonstrated in the E802A mutation. Although researchers are still investigating the underlying mechanisms of this resistance, a possible explanation is that mutations at 802 cause conformational changes to the RdRp active site that alleviate RMP-induced steric hindrance and thus allow for continued elongation of the RNA product strand [136].

Ribavirin

Ribavirin functions as a guanosine analog that produces broad-spectrum antiviral activity, primarily for hepatitis C and viral hemorrhagic fevers when used in conjunction with interferons (IFNs) (Fig. 12). Ribavirin monophosphate competes with inosine monophosphate (IMP) for binding to IMP dehydrogenase, which, when bound to IMP, synthesizes intracellular GMP and GTP [137]. The inhibition of IMP binding by ribavirin monophosphate leads to decreased GMP and GTP levels that, when low, indirectly inhibit RdRp enzyme activity by causing nucleotide imbalance. The downstream effects of this imbalance in turn interfere with mRNA capping, ribosomal activity, G-protein signaling, and other post-transcriptional processes [138].

Studies have demonstrated successful ribavirin activity against SARS-CoV replication *in vitro* when administered in combination with IFN-β [139,140]. However, ribavirin was shown to be ineffective against SARS-CoV *in vivo* [141]. When ribavirin and IFN-α-2a were used to treat patients infected with severe MERS-CoV, the treatment improved survival at 14 days but not at 28 days [142].

A retrospective study involving 115 patients with severe SARS-CoV-2 found that ribavirin therapy is not associated with improved health outcomes or

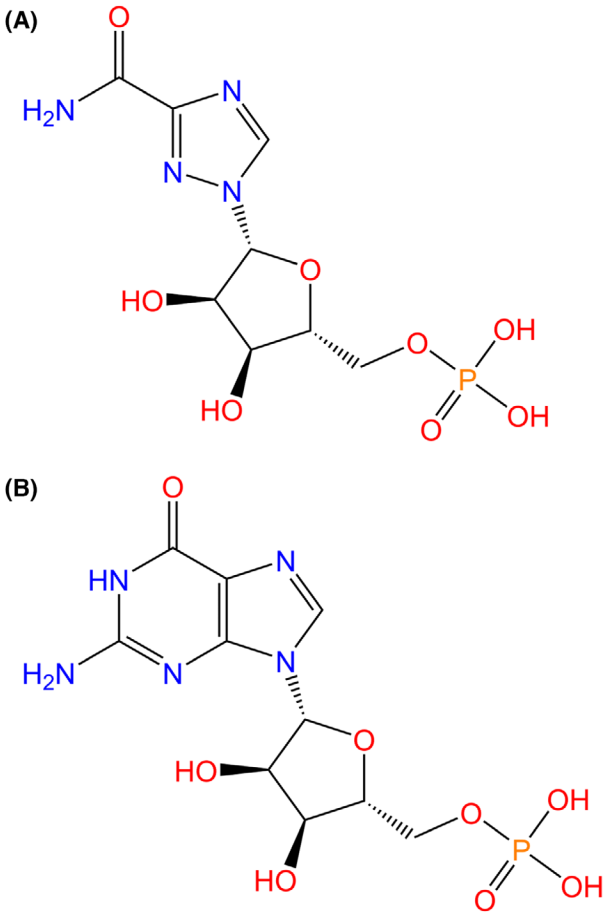

Fig. 12. Chemical structures of (A) ribavirin monophosphate and (B) GMP. Due to its function as a guanosine analog, ribavirin monophosphate is a broad-spectrum antiviral because it is able to compete with IMP for binding to IMP dehydrogenase, which in turn inhibits RdRp enzyme activity as a result of the creation of nucleotide imbalance [137,138].

mortality rates, with 17.1% of ribavirin patients and 24.6% of control group patients dying as a result of COVID-19 progression [48]. A larger, more recent retrospective study of 2037 COVID-19 patients confirmed the ineffectiveness of ribavirin/IFN-α in improving clinical health outcomes [143].

Prior to the aforementioned studies demonstrating the ineffectiveness of ribavirin-based therapies against SARS-CoV-2, the recommended dosage of ribavirin was 500 mg two or three times per day for no longer than a 10-day period, often in combination with IFN-α or lopinavir/ritonavir [144].

Favipiravir

Developed in 2014 as a potential treatment for influenza, favipiravir (also known as Favilavir) functions as a nucleoside analog and disrupts RdRp activity in SARS-CoV-2, similar to remdesivir and ribavirin [145,146]. Favipiravir undergoes phosphoribosylation upon entrance into the body, transforming favipiravir into its active form favipiravir-ribofuranosyl-5′-triphosphate (FRTP) [147,148]. Upon entrance into infected cells, FRTP mimics a substrate of RdRp. From there, FRTP incorporates itself into the viral RNA strand where it is mistaken for a purine nucleotide by RdRp (Fig. 13). Incorporation halts viral protein synthesis [150]. Favipiravir contains a strong binding affinity with RdRp, which causes a reduction in new viral RNA [151].

A study conducted in China compared the effectiveness of favipiravir and Arbidol with respect to shortening the recovery rate of individuals infected with SARS-CoV-2. No significant recovery rate differences were found between the two groups at day 7, but earlier resolution of fever and cough was seen in favipiravir-treated individuals [152]. A separate study carried out in Japan found over 80% of individuals with mild and moderate cases recovered after 14 days, whereas only 60% of COVID-19 patients with severe symptoms recovered following treatment with favipiravir [150]. Thus, favipiravir was concluded to be effective against non-severe COVID-19 but has subsequently been deemed non-essential for treatment of COVID-19 [80].

Favipiravir is an oral therapeutic that has an ability to treat many other RNA viruses. The typical treatment is 1800 mg twice a day for 1 day, then 800 mg twice a day for up to an additional 4 days. Common side effects of favipiravir include uric acid elevations, psychiatric symptom reactions, and increase of aspartate aminotransferase. The drug is already commercialized in several countries including Russia, Jordan, and Saudi Arabia [151].

Error catastrophe

Error catastrophe involves increasing the mutation rate in the viral genome to the extent of genomic instability, leading to the extinction of the virus. A notable mechanism in error catastrophe is tautomerization, where tautomers change DNA from their original form into their isomeric form. This reaction in therapeutics can cause mutagenesis in the creation of enzymes and thus makes the enzymes non-functional [153].

Molnupiravir

Initially considered a potential treatment for influenza virus, molnupiravir is an orally administered COVID-

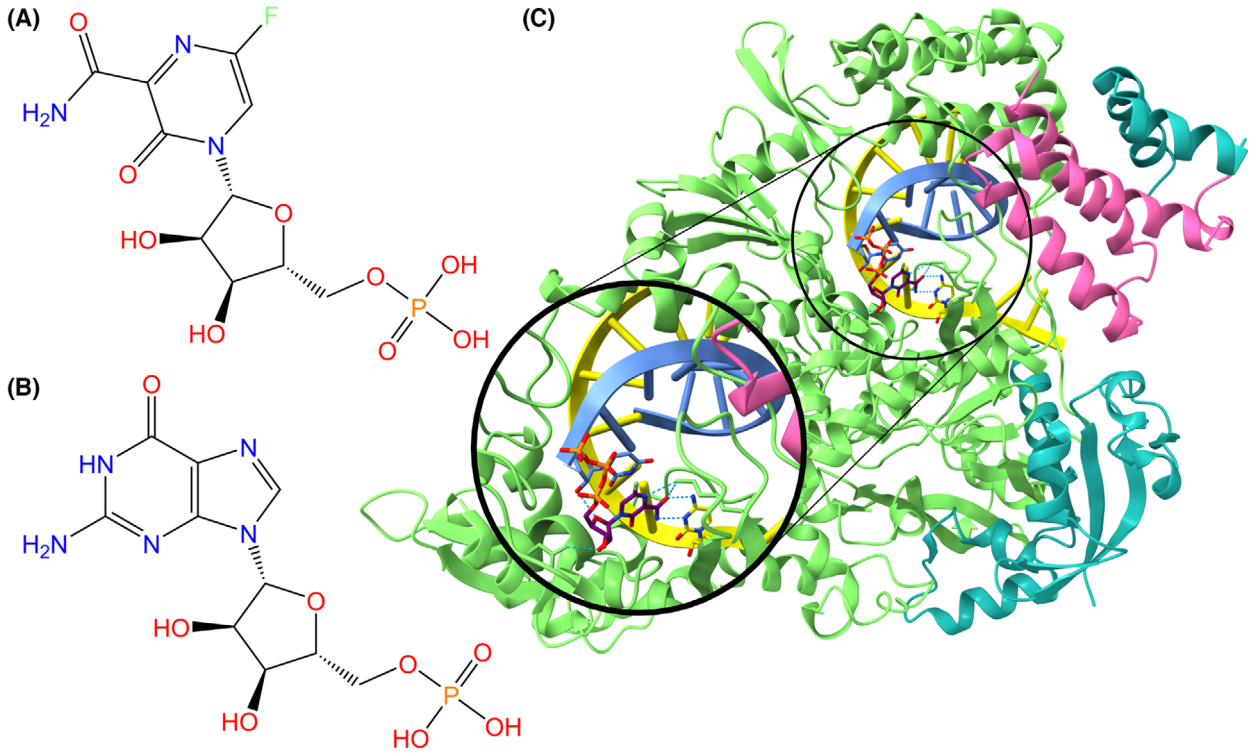

Fig. 13. Chemical structures of (A) favipiravir-ribofuranosyl-5′-monophosphate, a purine analog, and (B) guanosine monophosphate for comparison. (C) RdRp complex (NSP12: green, NSP7: pink, NSP8: teal) bound to template (yellow) and primer (blue) RNA strands and FRTP (purple backbone) (PDB ID: 7AAP, visualized with UCSF CHIMERAX, version 1.4) [148,149]. Hydrogen bonds between FRTP and RdRp or the RNA strands are represented by dotted cyan lines, and heteroatom colors correspond to those in the 2D visualization.

19 treatment developed by Drug Innovation Ventures at Emory University and later acquired by Merck and Ridgeback [153]. It was issued an EUA on 23 December 2021, for non-hospitalized patients experiencing mild-to-moderate COVID-19 in adults, but is notably not approved for children because of potential effects on bone and cartilage growth [81]. On 23 March 2022, the FDA extended molnupiravir's EUA to include COVID-19-positive adults for whom other FDA-approved treatments are not accessible [154].

Molnupiravir utilizes error catastrophe to inhibit RdRp through a two-step process. Upon entering the body, molnupiravir begins the process of mutagenesis. Molnupiravir enters the body as an isopropyl ester prodrug, where it is cleaved by the esterases of a host cell, forming the active nucleoside analog β-D-*N*4-hydroxycytidine (NHC), also known as EIDD-1931 (Fig. 14A,B). Active kinases convert the analog to NHC 5′ triphosphate (EIDD-1931-triphosphate). NHC-triphosphates are used as substrates for RdRp enzymes over cytidine and uridine triphosphates; adenine or guanine are then added to the active centers of RdRp to maintain RNA stability (Fig. 14C–E). The subsequent copies of RdRp are thus composed of these mutated RNA substrates that inhibit normal function. Molnupiravir is more prone to be an electron donor as opposed to an electron acceptor, which alters the conditions for viral infection [153,157].

In non-human primates, NHC has poor bioavailability and is rapidly metabolized in enterocytes. Molnupiravir prolongs antiviral effects by increasing the bioavailability of NHC. Molnupiravir has a wide therapeutic window with a quick onset of action. The drug has a half-life of 7 h and a maximum serum concentration of 13.2 $ng \cdot mL^{-1}$ [157].

Molnupiravir has been observed to target the upper respiratory tract. In a clinical trial using immunodeficient mice with human lung tissue, molnupiravir reduced the number of infectious particles in human lung tissue by 25 000-fold, with no adverse effects on the white blood cell or platelet counts. In a clinical trial with 741 patients with mild COVID-19 symptoms, molnupiravir significantly lowered the hospitalization rate of unvaccinated adults. A two phase, double-blind clinical study found that 1600 mg over 5.5 days is the most tolerable amount, with 800 mg taken twice a

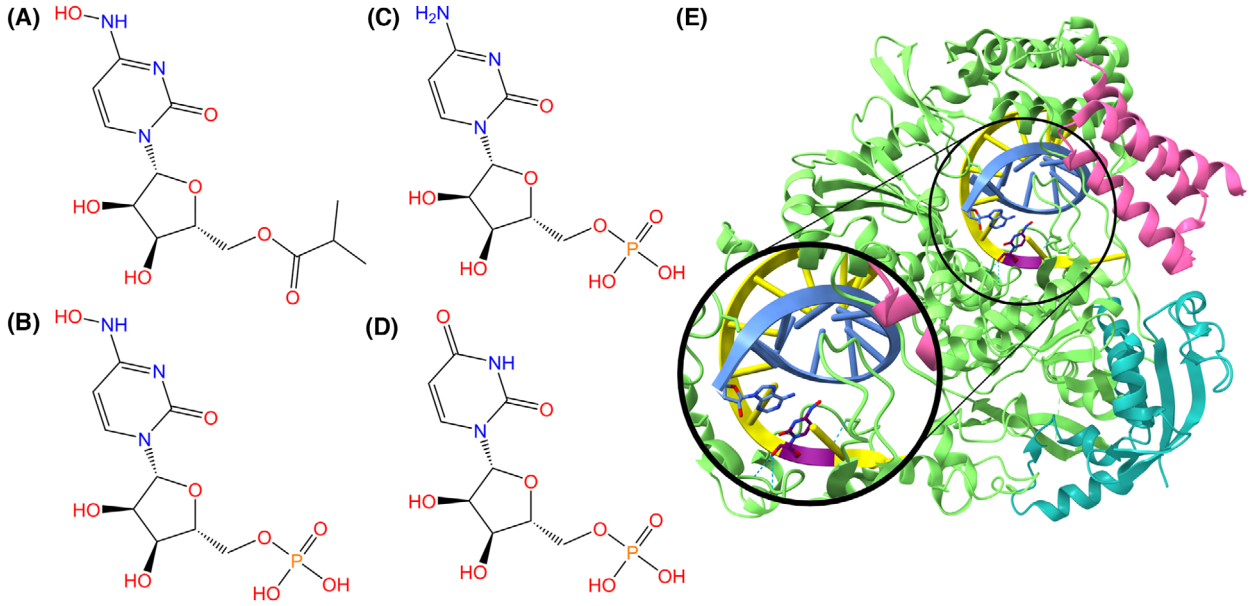

Fig. 14. (A) Chemical structure of molnupiravir, an isopropylester prodrug of β-D-*N*4-hydroxycytidine (NHC). (B) Chemical structure of NHC monophosphate. The active 5′-triphosphate form of the drug is incorporated into the RdRp and induces viral activity. (C) Chemical structures of cytidine monophosphate and (D) uridine monophosphate, which NHC/Molnupiravir imitates. (E) RdRp complex (NSP12: green, NSP7: pink, NSP8: teal) bound to template (yellow) and primer (blue) RNA strands. NHC (purple backbone) is incorporated in the template strand and is base paired with adenosine (PDB ID: 7OZU, visualized with UCSF CHIMERAX, version 1.4) [155,156]. Hydrogen bonds between NHC and RdRp or the RNA strands are represented by dotted cyan lines, and heteroatom colors correspond to those in the 2D visualization.

day. Molnupiravir showed a hospitalization risk of 7.1% compared to 14.1% with placebo. Additionally, the drug showed a significant decrease in hospital admissions and death by 50%; however, this was only observable in patients with mild cases of COVID-19. Current clinical trials in India do not show molnupiravir effectively treating moderate cases of COVID-19. As a result of its recent discovery, there are still many clinical trials testing the efficacy of molnupiravir [153]. Molnupiravir contains a risk of carcinogenesis although these observations were only at the cellular level. In phase 1 clinical trials, a greater proportion of participants reported more adverse effects with the placebo than molnupiravir (43.8% and 35.4%) [158]. Preclinical trials have also demonstrated the effectiveness of molnupiravir with respect to treating remdesivir-resistant viruses [158].

Few clinical studies have investigated the resistance of SARS-CoV-2 to molnupiravir. Because of molnupiravir's quick treatment, the viral error possibly blocks resistance, creating a high genetic barrier. In studies that exposed the influenza virus to molnupiravir multiple times, no allele dominant mutations or strong resistance were demonstrated. Another study exposed two lineages of MERS-CoV to increasing concentrations of NHC; however, there was little development of resistance [158].

Protein trafficking and post-translational modifications

Following cell entry, SARS-CoV-2 leverages host-cell ribosomes to create viral proteins necessary for replication. These viral proteins have become a target of multiple antiviral therapeutics, which are discussed in this section. Through either protein degradation or inhibition of intracellular transport, proper protein assembly is prevented, and subsequent viral functionality is lost. Here, we look at two antivirals that have shown some initial promise in the fight against COVID-19, but neither are approved for medicinal use against COVID-19 (Table 3). However, these therapeutics are unlikely to be used for COVID-19 treatment because of negative side effects (nitazoxanide) and lack of efficacy (ivermectin).

Nitazoxanide

Nitazoxanide (NTZ) is the first compound discovered in a class of antiviral agents known as thiazolides

Table 3. Summary of recommendations for protein trafficking and post-translational modifications targeted antivirals. Both antivirals in this section are not authorized for COVID-19 treatment.

Antiviral	Timing of treatment	References
Nitazoxanide	Not authorized for COVID-19 treatment	[159]
Ivermectin	Not authorized for COVID-19 treatment	[160]

[161]. The thiazolide class contains numerous nitazoxanide derivatives possessing antibacterial, antiparasitic, antiprotozoal, and antiviral properties [161–163]. Thiazolides were first developed and tested in the mid-1970s as an antiparasitic agent, but NTZ became increasingly popular in 1993 as an antiprotozoal treatment against *Cryptosporidium parvum* infections [164,165]. After being approved by the FDA in 2002 for treatment against *C. parvum*, NTZ has been explored as a remedy for numerous infections: influenza A, Ebola, hepatitis C, and MERS-CoV [166,167]. As such, the NIH instituted the first phases of trials for NTZ in August of 2020 as a potential treatment for COVID-19 [159]. Experiments and trials assessing the effectiveness of NTZ against COVID-19 in diverse populations are ongoing, and research continues to focus on both the potential mechanisms of action and efficiency of NTZ in a clinical setting [168–170].

NTZ is readily metabolized, after consumption, into its active metabolite tizoxanide (TIZ) (Fig. 15). TIZ forms via rapid deacetylation through hydrolysis by plasma esterases [171,172]. As a result of the versatility of TIZ, various mechanisms targeting viral entry, replication, and post-translational modification have been proposed and identified in the previously mentioned viruses [164,173–175]. There are various proposed mechanisms for TIZ in response to COVID-19, but three primary mechanisms exist: two regarding inhibition of viral entry inhibition and one involving inhibition of post-translational processing inhibition [168].

The first mechanism involves inhibition of the endosomal entry pathway of SARS-CoV-2 [176]. The S1 subunit of SARS-CoV-2 S protein exchanges disulfide bonds with ACE2 through a thiol-sulfide redox system, which is assisted by protein disulfide isomerase (PDI) activity, initiating endosomal entry. Consequently, inhibition of PDI prevents proper disulfide exchange, providing an inhibitory target for TIZ to act upon. TIZ allosterically inhibits PDI through S-nitrosylation, preventing proper association of the S1 subunit with ACE2 and subsequent viral entry [168].

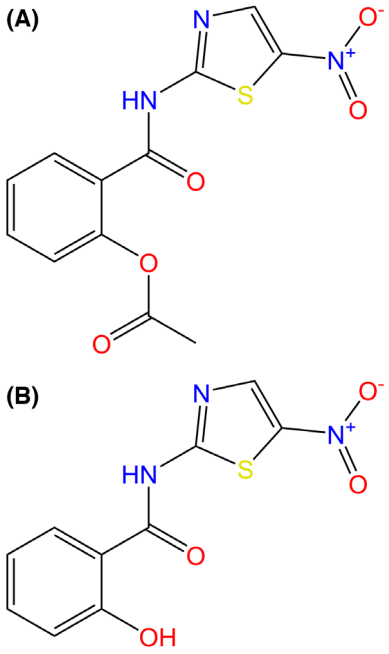

Fig. 15. (A) Chemical structures of nitazoxanide (NTZ) and (B) tizoxanide (TIZ), the active metabolite of NTZ, which is formed through rapid hydrolysis of NTZ's acetyl group by plasma esterases. NTZ and its derivatives have been found to have antimicrobial, antiparasitic, and antiviral properties. NTZ is theorized to have antiviral activity against SARS-CoV-2 by inhibiting viral entry and/or post-translational processing [161,168].

The second mechanism prevents non-endosomal entry of SARS-CoV-2. Furin and transmembrane-protease-serine-2, two host cell proteases, cleave S proteins, exposing the S2 subunit for fusion with the host cell membrane, allowing viral entry. The cleavage is dependent on thiol-disulfide exchange, catalyzed through PDI activity. Transmembrane-protease-serine-2 ectodomains, containing cysteine-rich residues, are regulated by PDI and provide disulfide exchange functionality. Similar to the endosomal mechanism, TIZ allosterically deactivates PDI via s-nitrosylation, preventing S2 exposure and viral entry [168].

The third involves post-translational processing inhibition of SARS-CoV-2, which also works through an autophagic mechanism. When the virus is infecting the host cell, AMP-protein activated kinases are inactivated, preventing autophagic degradation [168,177,178]. NTZ utilizes TIZ metabolites, which activate AMP-protein activated kinases by allosterically increasing the ratio of AMP bound to the γ regulatory subunit [173]. This in turn activates Unc-51-like kinase 1/2, leading to autophagosome formation and post-translational degradation of SARS-CoV-2 [179,180].

In vivo studies have been conducted to assess the COVID-19 symptom reduction ability of NTZ and

TIZ. One study of 392 patients (194 treated with NTZ, 198 treated with placebo) found no significant difference in the reduction of symptoms when comparing placebo and NTZ patients. However, secondary data showed statistically significant viral load reduction in NTZ-treated patients compared to placebo 5 days post-treatment [170]. A second study assessed symptom reduction in pregnant individuals, similarly, uncovering no significant correlation between treatment and symptom reduction [169]. Any adverse effects mostly parallel the symptoms of COVID-19 because NTZ has not shown clinical repression of symptoms [169,170].

The primary limitation of NTZ originates from the highly bound plasma membrane property of TIZ. Highly bound plasma membrane treatments penetrate cell membranes worse than minimally bound counterparts [181]. However, the high membrane affinity of TIZ limits combinatorial treatment prospects involving NTZ. One study assessing the compatibility of warfarin and TIZ found TIZ greatly reduced the outcompeted warfarin for membrane binding, inhibiting proper Warfarin function and potentially risking internal bleeding [182].

Ivermectin

Ivermectin is an antiparasitic drug derived from macrocyclic 22,23-dihydroavermectin B, which is obtained by bacterium in the *Streptomyces* genus (Fig. 16) [183]. Ivermectin was first commercialized to

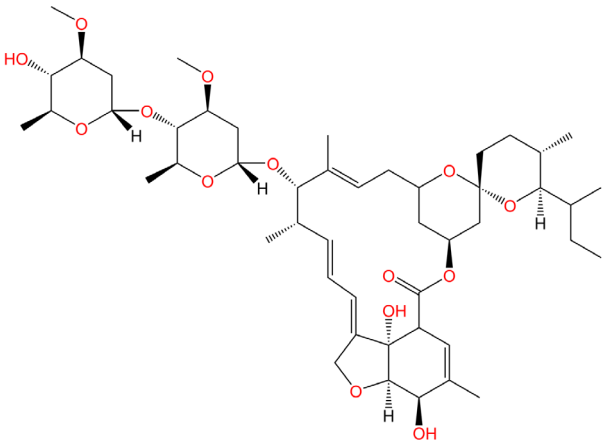

Fig. 16. Chemical structure of ivermectin. Ivermectin is commonly used as an antiparasitic drug for animals and humans. The drug has also demonstrated antiviral characteristics and as such was investigated for action against COVID-19 in the early stages of the pandemic, although the mechanism of action for these observed antiviral characteristics has yet to be determined [183].

treat parasitic illness in animals. In 1987, ivermectin would later be cleared for antiparasitic treatment in humans [184,185]. In recent years, ivermectin has also demonstrated antimicrobial activity against dengue fever, influenza, HIV, and other RNA viruses. The ability of ivermectin to inhibit viral reproduction promoted research on whether the same mechanism could work to combat SARS-CoV-2 [183,186].

The mechanism in which ivermectin works in stifling transmission of the COVID-19 is relatively unknown. The most accepted mode of action is via the suppression of importin α/β1 dependent transport of viral proteins into and out of the nucleus. Previous *in vitro* studies on ivermectin have indicated its ability to limit nuclear import of viral infections by binding to the armadillo complex on importins [186]. Blocking nuclear import of viral infections has been shown to inhibit virus proliferation of HIV-1 and dengue fever [187]. Other proposed mechanisms include competitive binding of ivermectin with the S protein of the virus, ionophore formation, anti-inflammatory effects, etc. [183,188].

There is conflicting information regarding the effectiveness of ivermectin for treating mild cases of SARS-COV-2. Some clinical trials earlier in the pandemic indicated a reduction in mortality rate and shorter recovery time post-infection after treatment with ivermectin [189]. However, the lack of standardized concentrations for ivermectin and standardized sample sizes for clinical trials at the time made it difficult to determine the efficacy of the drug for treating COVID-19 [160,184].

A significant number of recent studies have concluded that ivermectin has little to no effect on patient outcomes for SARS-CoV-2. In A 2020 double-blind study in Brazil, 1358 adults who had COVID-19 symptoms for at most 7 days were randomly assigned 400 $\mu g \cdot kg^{-1}$ of ivermectin for 3 days or a placebo. The conclusion of the clinical trial indicated that early treatment of ivermectin had no significant difference between the placebo and ivermectin group in terms of hospitalization rates (16.3% and 14.7%) and mortality rates (3.5% and 3.1%) [190]. Other recent clinical trials with the same dosage also concluded no apparent difference between ivermectin and the placebo group [186,190,191].

An increasing number of clinicals trials have indicated that the ivermectin is not effective in improving patient outcomes. In addition, adverse effects associated with ivermectin have also lowered the benefits of its usage. Applying ivermectin at concentrations above 150 $\mu g \cdot kg^{-1}$ can cause ataxia, nausea, and comas [186]. Overall, the adverse effects coupled with the lack

of clear efficacy of ivermectin has called into question its viability in treating SARS-CoV-2.

Immune response regulation

The treatments in this section focus on enhancing the body's natural immune defense system, rather than targeting the SARS-CoV-2 virus. The amplification of the body's response can better prepare the immune system to combat the negative symptoms that the virus will cause. Both drugs within this category target the inflammation response to improve COVID-19 outcomes. They both have shown initial promise, although as of yet there is insufficient clinical evidence to allow treatments to be approved in the fight against COVID-19 (Table 4).

Interferons

Consisting of three types (I, II, III), IFNs are cytokines naturally secreted by the body upon viral infection. IFNs act by binding to cell-surface receptors and activating the Janus kinase signal transducer and activator of transcription via receptor tyrosine kinases [194]. This regulates the transcription of genes related to innate and adaptive immunity, which can increase an inflammatory response and drive immune cell differentiation to fight infection [195].

Interferon immune response properties have prompted research to determine the efficacy of IFNs as a therapeutic option for COVID-19. Of the three types, type I (IFN-β-1a and IFN β-1b) has been studied most extensively and show the best results in improving COVID-19 outcomes (Fig. 17). Type II (IFN-γ) has been cleared for safety, although more research is needed to determine their efficacy. Type III has not been cleared and the literature points to inconclusive data regarding its efficacy [198].

Type I IFNs are secreted by cells upon viral infection and stifle the proliferation of the virus. Type 1 IFNs act by binding to a heterodimer of IFN-α receptors 1 and 2. Binding of the type I IFN leads to the phosphorylation of Janus kinase and tyrosine kinase, which phosphorylates the tyrosine residues on the heterodimer. Phosphorylation of the tyrosine residue recruits signal transducers and activators of transcription (STAT 1 and STAT 2). STAT1 and STAT2 are activated to form a homodimer and interact with IFN regulatory factor 9 resulting in IFN-stimulated gene factor 3. IFN-stimulated gene factor 3 is translocated to the nucleus to induce the genes that are controlled by IFN-stimulated response elements, which leads to antiviral gene expression [194].

IFN-β-1a has been administered via intravenous, inhalation, and subcutaneous means and shows promise in improving patient outcomes for COVID-19 when applied in the early stages of infection [198]. In a double-blind clinical trial where 100 adults with COVID-19 were randomized to receive either a placebo or IFN-β-1a via a nebulizer for 14 days, three of the placebo group died during the study, whereas no deaths occurred for the experimental group. The experimental group also experienced a faster recovery rate compared to the placebo group [199]. Although there are promising data on the efficacy of IFN-β-1a, late application of IFN-β-1a contributes little to no effect on improving patient outcomes [200]. In a randomized clinical trial, 446 COVID-19 patients with an age range of 8–96 years were administered IFN-β-1a, and the early treatment group, late treatment group, and placebo had a recovery rate of 99.1%, 84.6%, and 95.1% respectively [200]. Studies have also revealed that IFN-β-1a may lead to an increase in acute

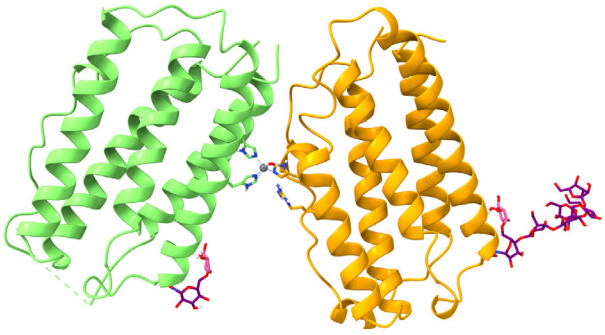

Fig. 17. Structure of an interferon-β dimer (PDB ID: 1AU1, visualized with UCSF CHIMERAX version 1.4) [196,197]. Interferons are natural cytokines, which regulate adaptive and innate immune responses [194]. The two monomer chains (orange and green) of the interferon-β dimer are coordinated by a zinc molecule (gray, with metal coordination bonds in lavender). Both chains possess one or more beta-D-glucopyranose residues (purple backbone) and one 6-tert-butylsulfonyl-*N*-(5-fluoro-1H-indazol-3-yl) quinolin-4-amine residue (pink backbone).

Table 4. Summary of recommendations for antivirals targeted towards immune response regulation in response to COVID-19 infection. Both antivirals in this section are not authorized for COVID-19 treatment.

Antiviral	Timing of treatment	References
Interferons	Not authorized for COVID-19 treatment	[192]
Dexamethasone	Not authorized for COVID-19 treatment	[193]

respiratory distress, hypersensitivity, and neurological illness in some patients. However, patients did experience increased discharge rates and decreased morbidity from the IFN-β-1a treatment [201]. IFN-β-1b shares functions with IFN-β-1a. Studies have shown that IFN-β-1b is safer than IFN β-1a, but more research is needed to determine its efficacy [198]. The emergence of robust therapeutics for COVID-19 coupled with the potential side effects of IFN-β-1a has hampered the drug's potential utilization for treating COVID-19.

Dexamethasone

Dexamethasone functions as a corticosteroid medication and has been of interest in the fight of COVID-19 as a result of its use treating rheumatoid arthritis, asthma, different types of cancers, and systemic lupus erythematosus (Fig. 18) [202]. Corticosteroids are a class of medications that act as an immunosuppressant. The relative abundance of dexamethasone is consistent around the globe because of its status as an Essential Medication by the WHO, and this further ensures its accessibility [203].

The corticosteroid reacts with receptor proteins in the cytoplasm, creating a steroid-receptor complex, which then enters the nucleus and acts by inhibiting the transcription of mRNA to downregulate target protein synthesis [204]. Dexamethasone alleviates the inflammation that can occur with COVID-19 infection by slowing the migration of immune cells to infected areas and reducing immune cell chemotaxis (through inhibition of cytokine release) and vasodilation [205].

Clinical trials testing the use of dexamethasone dosed patients at 6 $mg{\cdot}day^{-1}$ for 10 days. One study conducted by the Recovery Collaborative Group based at Oxford University found that the risk of mortality in the experimental group was significantly lower compared to the control group (21.6% compared to 24.6%). They also found that outcomes improved when patients underwent mechanical ventilation (40.7% compared to 29.0%) or just non-invasive oxygen (25.0% compared to 21.5%) [206].

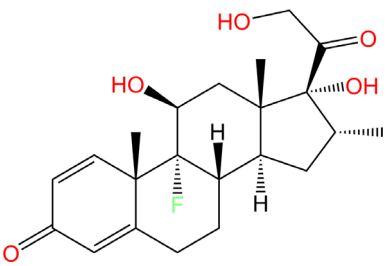

Fig. 18. Chemical structure of dexamethasone, a corticosteroid that acts as an immunosuppressant and reduces inflammation. Dexamethasone is used to treat rheumatoid arthritis, asthma, some cancers, and systemic lupus erythematosus and was investigated as a treatment to improve COVID-19 patient outcomes in the early stages of the COVID-19 pandemic [202].

Corticosteroid resistance may arise through the course of diseases that cause inflammation. Multiple proposed mechanisms of resistance include reduced steroid-receptor complex translocation into the nucleus, competition for corticosteroid binding between glucocorticoid receptors α and β, where glucocorticoid receptor-B inhibits glucocorticoid receptor-A activity, and increased secretion of macrophage migration inhibitory factor [207]. These resistances pose a large problem to populations that have chronic inflammation, namely in asthmatics and smokers. However, few studies have been conducted specifically looking at resistance pertaining to the use of dexamethasone in COVID-19 patients.

Conclusions and perspectives

As the global community has raced to develop and improve vaccines in response to the COVID-19 pandemic, the need for antiviral therapies has become more pressing. Antiviral therapies present major benefits in the fight against the COVID-19 pandemic, including ameliorating symptoms in mild to moderate COVID-19 cases by providing a mechanism for preventing successful viral infection of a cell, with the potential to save thousands of lives. Successful drugs improve outlooks for both vaccinated and unvaccinated individuals, mitigating symptoms, decreasing mortality, and increasing discharge rates. Currently existing therapeutics that have been shown to be effective have a wide range of mechanisms targeting various points in the COVID-19 maturation cycle (Table 5).

Of the drugs mentioned in this review, molnupiravir, Paxlovid, Evusheld, sotrovimab, REGEN-COV, BNE, and bebtelovimab have all been granted EUAs for SARS-CoV-2 treatment [23]. Paxlovid holds the most promise because of its higher efficacy and oral administration compared to other approved drugs. Accordingly, governmental bodies in the USA have invested heavily in this treatment, purchasing 10 million treatment courses of Paxlovid [22]. Paxlovid is highly effective because of its close imitation of the amino acid sequence found at splice sites in SARS-CoV-2 polyproteins, which allows it to inhibit the viral protease that processes the proteins required for viral genome replication. However, as an oral inhibitor, Paxlovid does not confer immunity against COVID-19 to patients

Table 5. Summary of antiviral therapies and the category of the virus life cycle that they target. Therapies marked with a single asterisk (*) are authorized for use by the FDA in the USA under an EUA as of 31 July 2022. Therapies marked with two asterisks (**) were once approved under an EUA but are not currently authorized for treatment in the USA because of the prevalence of the Omicron variant of SARS-CoV-2. The therapy marked with three asterisks (***) has been fully approved by the FDA for the treatment of SARS-CoV-2, which supersedes the therapy's original EUA. Therapies without asterisks have not been authorized for the treatment of COVID-19 temporarily or permanently by the FDA.

Stage of virus maturation	Antiviral therapy targets	References
Viral entry into host cell	Evusheld*, REGEN-COV**, bamlanivimab and etesevimab**, bebtelovimab*, sotrovimab**, Arbidol, nitazoxanide, chloroquine	[8,23,33,44,48,50,59,63,65,66,168]
Viral replication	Paxlovid*, Kaletra, remdesivir***, favipiravir, ribavirin, molnupiravir*	[90,92–96,119,127,138,150,153,157]
Protein trafficking and post-translational processing	Nitazoxanide, ivermectin	[168,183,186,188]
Immune response regulation	Interferons, dexamethasone	[194,205]

[90,95,96]. Of the monoclonal antibodies discussed, Evusheld is most promising as a result of its authorization for use as a preventative treatment against contracting COVID-19 and experiencing severe symptomatic illness [9]. A preventative treatment would provide a greater form of defense against COVID-19 infections because individuals would no longer have to rely on the post-infection therapeutic techniques. Post-infection treatment with bebtelovimab is advantageous because its effectiveness has been demonstrated on all known variants of concern (as of August 2022), although bebtelovimab has a lower efficacy rate than Paxlovid and Evusheld [51].

As the world is now past its second anniversary subsequent to the onset of the COVID-19 pandemic, these antiviral therapies provide hope for a future without the fear of SARS-CoV-2. Research and development are paramount in the continued fight to save lives and improve future pandemic responses. More techniques should continue to be investigated, both as preventative treatments and to ameliorate symptoms after infection. Importantly, more extensive investigation of therapeutic techniques for severe COVID-19 cases is necessary, given that such cases pose the greatest risk of life-threatening symptoms. This review aptly summarizes the state of COVID-19 therapeutics as they stand and provides insight into the areas that require further investigation.

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Author contributions

DKB, ARG, LH, AAH, ALL, OGP, BAR, TTS and KTV reviewed the literature, wrote the manuscript, and revised the manuscript. DKB, ARG, LH, AAH, ALL, OGP, BAR, TTS, and KTV contributed equally to this manuscript. TPC carefully revised and co-wrote the manuscript.

Conflicts of interest

OGP is currently participating in an unpaid internship at AstraZeneca as of Fall 2022 until Spring 2023. The remaining authors declare that they have no conflicts of interest.

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